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ROOT DEFECTS AND FUNGI ASSOCIATED WITH THE LITTLE-LEAF DISEASE OF SOUTHERN PINES

L. W. R. JACKSON[1]

(Accepted for publication September 20, 1944)

INTRODUCTION

A serious deterioration and dying of southern pines, particularly shortleaf pine (*Pinus echinata* Mill.), known as the little-leaf disease, was first reported from Alabama in 1935. Extensive surveys (11, 12) have shown that little leaf is widely distributed through much of the Piedmont region from central Virginia to western Alabama, and through the upper coastal plain of Alabama to northeastern Mississippi. The disease occurs extensively on shortleaf pine, and to a lesser extent on loblolly pine (*P. taeda* L.), Virginia pine (*P. virginiana* Mill.), and pitch pine (*P. rigida* Mill.), and symptoms resembling little leaf have been found occasionally on longleaf pine (*P. palustris* Mill.). Typical little leaf has seldom been found on trees younger than 20 years or less than 3 inches in diameter at breast height.

The symptoms on the aboveground parts of affected trees are reduced needle length, varying degrees of yellowing, and a shortening of the twig internodes, which give the crown a sparse, tufted appearance.[2] When branch dying occurs, it usually progresses upward from the base of the crown. Another characteristic of little-leaf trees is a marked slowing down of diameter growth. Outer rings are often greatly reduced in width (2, 12). No infections related to little leaf and caused by pathogenic organisms have been found on the aboveground parts of diseased trees.

The root systems of shortleaf pines affected with little leaf are consistently more defective than those of healthy trees. There is an excessive exfoliation of bark, producing brown patches, dieback of the fine roots, and large, pitchy, cankerlike lesions.

The cause of the little-leaf disease has not been determined. The work on the cause has been based chiefly on three hypotheses, namely, that little leaf results from (1) a root deterioration caused by pathogenic fungi, (2) a virus, or (3) physical and chemical soil factors. This paper presents work on the characteristics of the various root defects, the condition of the mycorrhizae, and the pathogenicity of two fungi found associated with root defects. Shortleaf pine was the only host used in the experimental tests.

[1] Associate Pathologist, Division of Forest Pathology, United States Department of Agriculture.

[2] A detailed description of symptoms will appear in a forthcoming U. S. Dept. Agr. Circular entitled "Little-leaf disease of pine" by G. H. Hepting, T. S. Buchanan, and L. W. R. Jackson.

ROOT DEFECTS

General Characteristics of the Root Systems of Diseased and Healthy Shortleaf Pines

The condition of roots of diseased and healthy trees was examined in detail by washing out the root systems of 3 diseased and 4 healthy trees with a high-pressure stream of water delivered by a force pump. Samples of the feeding roots[3] were collected from each tree for an analysis of the amount of dieback and the condition of the mycorrhizae.

The root excavation work showed no observable difference in the distribution, extension, or gross morphology of the primary and larger lateral roots and the taproots of the diseased and healthy trees. On the diseased trees, the roots of all sizes, including feeding roots, were usually covered with a thick layer of brown patch. On the healthy trees, the large roots were covered with brown patch but the roots less than one inch in diameter usually had only small and widely scattered brown patches. Dead primary and lateral roots were common on little-leaf trees. Diseased trees had

TABLE 1.—*Amount of dieback of feeding roots on diseased and healthy shortleaf pines*

Trees and roots examined	Condition of trees	
	Healthy	Diseased
Total number of trees	4	3
Total number of live feeding roots examined	775	679
Average live length (inches) of feeding roots with dieback	2.4	2.8
Percentage of feeding roots with dieback	17	71

many more feeding roots that were dead or were dying back from the ends than had healthy trees.

The analysis of the condition of the feeding roots was based on samples of 139 to 281 feeding roots per tree from 4 healthy trees, and from 122 to 332 feeding roots per tree from the 3 diseased trees. Little-leaf trees had about 4 times as many killed-back feeding roots as did healthy trees (Table 1). In both diseased and healthy trees the feeding roots with dead ends had an average live length of 2 to 3 inches. These results, together with field observations, have shown that the bulk of the feeding roots on diseased trees are usually short and stubby, sometimes reduced to mere spurlike structures, and have from one to several lateral replacement roots. The dieback of feeding roots, such as occurs on a little-leaf tree, must greatly reduce the absorbing capacity of the root system.

The cause of the excessive dieback of feeding roots on little-leaf trees has not been determined. A certain amount of root decadence or dieback occurs normally on pines after the root system has fully developed. According to Büsgen and Münch (3), the lateral roots of healthy pines attain their

[3] In this paper, the term feeding root is used for the small mycorrhiza-bearing lateral roots, which are usually less than one-tenth of an inch in diameter.

greatest spread in youth or up to about 14 years. They also state that the cessation of root elongation is accompanied by extensive dying of the ends of the roots and the replacement of the dead parts by lateral roots of a higher order. In working with pitch pine and shortleaf pine in New Jersey, McQuilkin (7) found that the stronger primary lateral roots ceased to elongate after the trees attained a height of 25 feet and a diameter of 4 inches. Although a certain amount of root decadence occurs normally on healthy adult pines, the dieback of the feeding roots on little-leaf trees is regarded as an important symptom. In working with Scotch pine (*Pinus sylvestris* L.), Laitakari (6) also found that some dead root ends occurred on vigorous trees, but the presence of many dead root ends indicated that the root system was retarded.

Condition of the Mycorrhizae

Root analysis showed no observable difference in either the condition or the abundance of mycorrhizae on diseased and healthy shortleaf pines. All of the mycorrhizae examined were richly branched and bifurcate, and grew as coralloid clusters. Mycorrhizae of diseased trees were always well developed and appeared normal if the feeding roots were healthy.

Characteristics of Brown Patch on Roots of Diseased and Healthy Pines

Histological study shows that the brown patches consist of older layers of the secondary phloem (Fig. 1, A), which have abscissed by the development of periderm layers. They are light brown when first cut off, but turn dark brown with age. The size and arrangement of the elements of the brown patches did not differ from those of the underlying live phloem, indicating that no pathological changes had occurred during their formation. Dark hyphae that closely resemble those of a *Torula* are abundant throughout the brown-patch layers.

Brown patches are irregular in outline and vary from minute spots to areas of several square inches. On healthy trees, the brown patches are widely scattered but they may encircle the root in localized areas. On severely diseased trees, however, most of the roots, including the feeding roots, are usually covered with a thick layer of brown patches, which give them a rough scabby appearance. Though variable in depth, these patches involve half or more of the phloem (Fig. 1, A). Brown patches are often much deeper on some of the poorer roots of little-leaf trees, sometimes extending to the current sieve-tube layer. In such extreme cases the secondary phloem is reduced to a thin white film, consisting of only the current sieve-tube layer. When pried out with a knife, the brown patches rupture along the periderm layer and leave a conspicuous depression in the bark.

An excessive exfoliation of root phloem, such as occurs in little-leaf trees, decreases the food-storage capacity of the root system. Microscopic examination of root and trunk bark of shortleaf pine collected in February

showed that brown-patch layers were always devoid of starch. In the living portion of the bark, the parenchyma cells in the secondary phloem, including the current sieve-tube layer (Fig. 1, B), were always full of starch grains. Starch was abundant in the epithelial cells surrounding the resin ducts

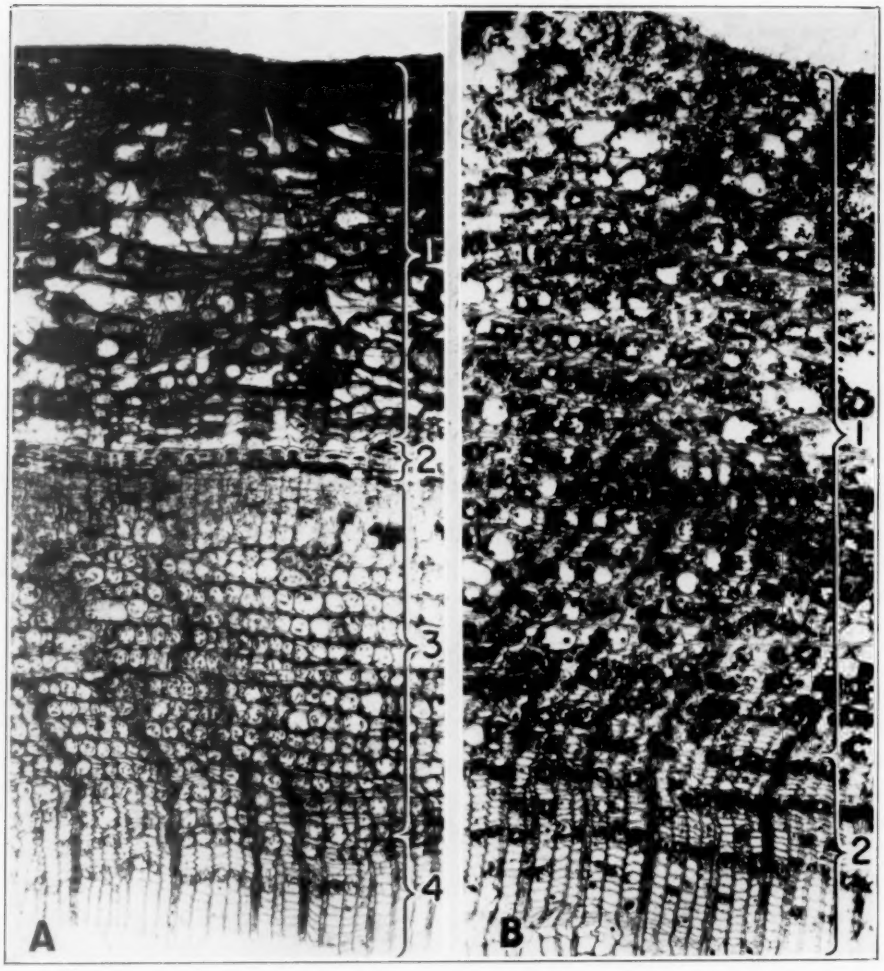

FIG. 1. Photomicrographs of shortleaf pine root bark. A. Transverse section showing (1) brown patch, (2) periderm layer, (3) secondary phloem, and (4) current secondary phloem layer. B. Transverse section showing accumulation of starch grains in (1) old secondary phloem and in (2) current secondary phloem layer. Starch grains stained black with iodine.

and in the medullary rays. Only a trace of starch was observed in the tissues of the trunk bark. Root bark is thicker than trunk bark and has larger and more abundant parenchyma cells for storing starch.

In both the root and trunk bark, the parenchyma cells (Fig. 1, A and B) used for storage arise in tangential rows in the sieve-tube layer and then

increase in size as they are pushed outward by new growth, forming closely compacted rows between narrow bands of crushed sieve-tube layers. The secondary phloem of pine roots is an important reservoir for the storage of reserve food. Though little information is available on the use of the starch stored in pine roots, it is suspected that a large part of it is used for the growth and development of the root system. Regardless of the ultimate use of the root foods, it appears that the food storage capacity would be decreased if excessive brown patch developed at the expense of the storage tissue beneath.

A survey (Table 2) showed that the roots of diseased shortleaf pines had nearly 6 times as much brown patch as the healthy shortleaf pines within the little-leaf range, and 32 times as much brown patch as the healthy shortleaf pines growing outside the range. Brown patches were found on roots of healthy loblolly and longleaf pines within the little-leaf range, and on Virginia pine outside the range, but the percentage of root bark affected

TABLE 2.—*The occurrence of brown patch on the roots of southern pines*

Locality[a]	Species	Condition of trees	Trees	Roots	Roots with brown patch	Average diam. roots	Area with brown patch	Age of trees
			No.	*No.*	*No.*	*Inches*	*Per cent*	*Years*
S. C., Ala.*	Shortleaf	Little leaf	22	43	43	1.8	96	31
S. C., Ala.*	Shortleaf	Healthy	23	45	31	1.5	17	29
Ga., Miss.	Shortleaf	Healthy	39	78	32	1.6	3	47
S. C., Ala.*	Loblolly	Healthy	10	20	5	1.7	7	29
Miss.	Loblolly	Healthy	9	18	3	1.5	1	34
Ala.*	Longleaf	Healthy	9	17	5	1.6	14	36
Ga.	Virginia	Healthy	5	10	5	1.5	4	45

[a] Asterisk indicates localities within the known range of little-leaf disease. All others were outside the range.

was small, being 14 per cent or lower. The amount of brown patch was somewhat larger on healthy loblolly and shortleaf pines growing in little-leaf localities than on the same species growing in healthy stands outside the little-leaf range. It is the abundance rather than the presence of brown patch on the roots of pines that must be regarded as an important symptom of the little-leaf disease.

Brown patch is regarded as the excessive and premature formation of rough bark. The cause of the larger amount of brown patch on the roots of little-leaf trees is not definitely known. Though little information is available on the histology of pine bark, it is possible that the initiation of brown patch results from the devitalization of the older part of the phloem layer. Such devitalization may result from aging, the effects of pathogenic organisms, a deficiency of essential elements, or the accumulation of toxic substances.

Pitch Cankers on Roots

Large, pitchy, cankerlike lesions have been observed frequently on the primary and lateral roots of healthy and diseased shortleaf pines, but

mostly on diseased trees. Siggers and Doak (11) also reported that they found roots with black masses of pitch and soil adhering to the bark, and with the underlying woody part of the root heavily infiltrated with resin. Pitch cankers are from a few inches to about a foot long, depending on the

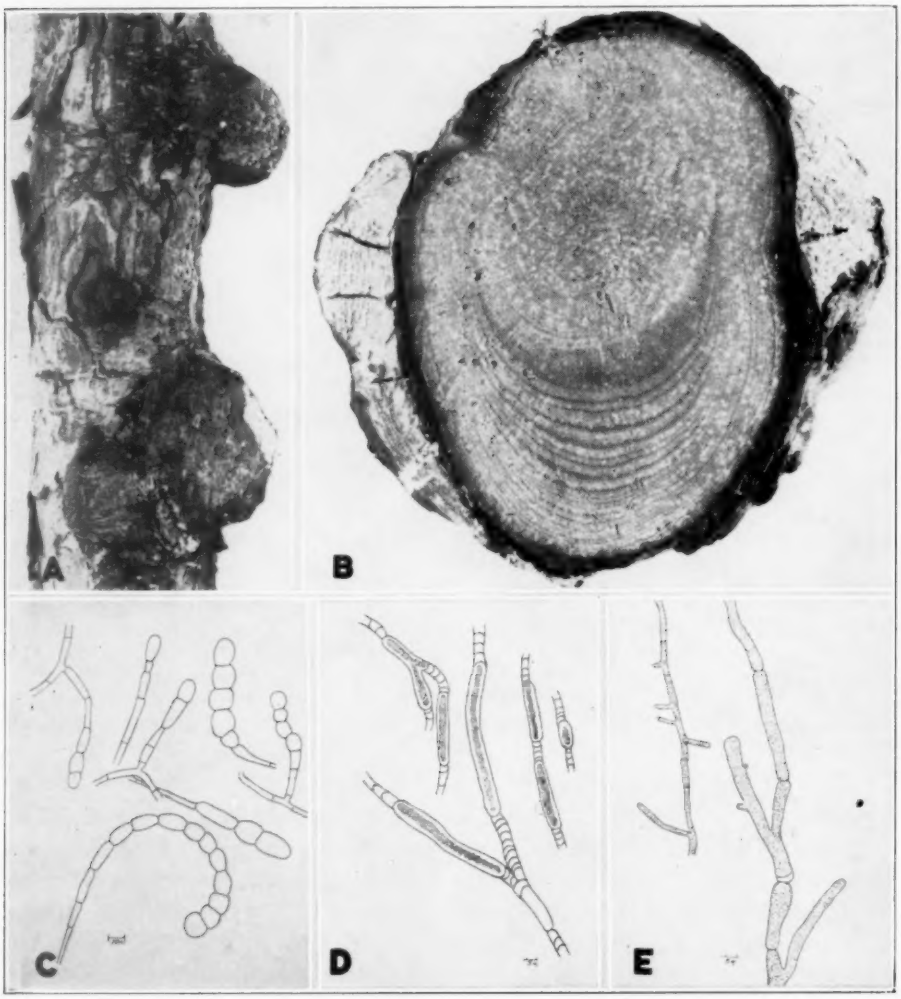

FIG. 2. Photographs showing characteristics of fungi associated with shortleaf pine roots. A. External appearance of tuckahoes of *Poria cocos*. B. Transverse section showing internal structure of tuckahoes of *Poria cocos*. C. Conidia and conidiophores of *Torula marginata*. D. Chlamydospores and heterocyst-like cells of *Poria cocos*. E. Small and large hyphae of *Poria cocos*.

size of the root. The bark is deeply cracked and the underlying wood is infiltrated with pitch, the extent of infiltration varying from a few rings to the entire stele in extreme cases. These lesions are usually covered with large masses of pitch-infiltrated soil firmly cemented to the bark. The soil masses are covered with dark hyphae resembling those of the *Torula* found in the

brown patches and giving them a dark sooty appearance. Detailed studies of root defects have not progressed far enough to offer any conclusions regarding the cause and significance of the pitch cankers.

FUNGI

Occurrence of Tuckahoes on Roots of Diseased and Healthy Shortleaf Pines

Root excavations showed that tuckahoes occur frequently on roots of diseased shortleaf pines but they have not been found on the roots of healthy trees. Tuckahoe is a common name of the sclerotium of a soil-inhabiting fungus known as *Poria cocos* Wolf. Isolations from the tuckahoes on shortleaf pines yielded a fungus that has been identified as a strain of *Poria cocos*. The tuckahoe fungus has not been isolated directly from such root defects as pitch cankers and dieback of the feeding roots.

The tuckahoes (Fig. 2, A) on roots of diseased pines vary from one-half to two inches and appear as irregular lumps on the surface of the bark. A fresh sclerotium (Fig. 2, B) is a compact, white mass of fungus cells with a cheesy consistency, striated with thin layers of bark, which becomes hard and fissured when dry. The tuckahoes (Fig. 2, A) on the roots of little-leaf trees differed markedly, both in size and shape, from the large tuberous tuckahoes that have been described previously (13, 15, 16).

Fungi Associated with Brown Patch

A species of *Torula* was consistently isolated from brown patches on roots of diseased shortleaf pines as well as roots of healthly shortleaf, loblolly, longleaf, and Virginia pines in the little-leaf belt. The *Torula* was isolated also from recently formed brown patches on healthy shortleaf, loblolly, longleaf, and Virginia pines growing outside the present known range of the little-leaf disease. This brown patch *Torula* is apparently widely distributed on the roots of pines in the Piedmont.

In 1939, Siggers[4] isolated a fungus from roots of little-leaf and healthy pines in Alabama, and from diseased roots of slash pine (*Pinus caribaea* Morelet) seedlings in a forest-tree nursery in Mississippi. This fungus has the same cultural characteristics as the brown-patch *Torula*.

Cultural Characteristics of the Brown-Patch Torula

The brown-patch *Torula* is more closely related to *Torula ligniperda* (Willk.) Sacc. (9, pp. 565–566; and 10) than to any of the other species of *Torula* described by Saccardo (9). The brown-patch fungus differs from *T. ligniperda*, as shown by comparisons with an authentic culture and a description by Siggers (10) of *T. ligniperda*, in having: (1) larger conidia, (2) longer chains of conidia, (3) a sharply defined white margin of the mycelium, and (4) a more compact and faster-growing mat of mycelium.

[4] P. V. Siggers, Associate Pathologist, U. S. Dept. of Agr., Bureau of Plant Industry, Soils and Agricultural Engineering, Division of Forest Pathology, Saucier, Mississippi.

On the basis of these differences, the brown-patch fungus is described as a new species of *Torula*. The characteristics of the conidia are shown in figure 2, C.

Torula marginata sp. nov.

Mycelium (on potato-dextrose agar) black with white margin, floccose in central part, appressed at outer part, growth very slow, with numerous black flecks in agar; mats fractured radially; hyphae 2–4 μ wide, dark, septate, without clamps, occasionally anastomose, walls sometimes roughened; conidia catenate, 2–12 or more but mostly 6 in a chain, oblong to (becoming) spherical, thick-walled, 1-guttulate, dark-brown, smooth, 12–13 ×11–12 μ, on short simple conidiophores, often appearing intercalary by the germination of the terminal conidium.

Habitat. Associated with dead bark on roots of *Pinus echinata, P. taeda, P. palustris,* and *P. virginiana* in the southeastern United States. Type specimen, Forest Pathology No. 90914, deposited in Mycological Collections, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

Coloniae[5] in agaro solani-dextroso atrae margine distincto albo, centro floccosae, margine appressae, tarde crescentes, radiatim fringentes, ex hyphis 2–4 μ latis fuscis septatis non fibulatis interdum anastomosantibus saepe verrucosis compositae; puncta numerosa nigra in agaro dispersa; conidia in catenulis cellularum 2–12 vel plurium plerumque 6 disposita, oblonga usque spherica, ex extremo proximo ad distantem gradatim magnitudine crescentia, crasse tunicata, 1-guttulata, atro-fusca, levia, 12–13 × 11–12 μ, in conidiophoris brevibus simplicibus orta; conidia terminalia catenularum submersarum germinantia et hyphas producentia ut catenulae intercalares videantur.

Hab. cum cortice emortuo consociata in radicibus *Pini echinatae, P. taedae, P. palustris,* et *P. virginianae,* in regione austro-orientali, U. S. A.

Cultural Characteristics of the Tuckahoe Fungus

The fungus isolated from tuckahoes on pine roots is regarded as a strain of *Poria cocos*. Its growth on potato-dextrose or 2 per cent malt agar was rapid and uniform, the fungus forming 90-mm. mats in 3 days at 26° C. The mycelium is cottony and white in 10-day-old cultures but becomes appressed and frequently is tinged with some shade of brown with age. The mats are soft, friable, and odorless, and they do not discolor the media. All of the cultures were white when first isolated. The mycelium is fragrant when grown on large quantities of cooked wheat. The fungus usually produces lacerate-poroid, white, resupinate sporophores on 10-day-old mats in plates and tubes. These sporophores produce viable basidiospores. The mycelium is composed of small and large hyphae (Fig. 2, E). The small hyphae are 2–4 μ in diameter, hyaline, thin-walled, septate, and without clamps. The large hyphae are 7–12 μ in diameter, hyaline when first formed, but turn to pale yellow with age, septations slightly constricted, and without clamps. In old cultures, the large hyphae (Fig. 2, D) are transformed into long, thick-walled, pale yellow chlamydospore-like cells that are separated by rows of narrow, empty heterocyst-like cells. The length of the chlamydospore-like cells is variable but the average size of 10 cells from each of 3 different isolates was 52–71 × 7–12 μ.

An occasional culture developed a dark-brown mat. The dark-brown cultures always reverted to the typical white form when replanted on agar.

[5] The Latin description was prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

The mycelium is appressed and white at first, soon turning to cinnamon-rufous[6] or chestnut-brown and becoming fragrant. The medium changes to dark brown. The dark-brown form of the mycelium can be produced by crossing different isolates but not by crossing different cultures of the same isolate. A zone of dark-brown mycelium forms across the plate along the juncture of the colonies.

The morphological features of the *Poria* isolated from tuckahoes on roots of little-leaf trees are comparable with those given for *Poria cocos* by Davidson, Campbell, and Vaughn (5), Wolf (15), and Weber (13). Variations in the color of the mycelium of the little-leaf isolates and the isolates reported by other investigators (5, 13, 15) are regarded as strain differences. The formation of chlamydospore-like cells, fragrance of mycelium on cooked wheat, and the dark-brown form of growth are new features for *Poria cocos*.

INOCULATIONS

Pathogenicity of Torula marginata and Poria cocos on Roots of Adult Healthy Shortleaf Pines

Primary roots of shortleaf pines 4 to 6 inches in diameter at breast height were inoculated, in situ, with *Torula marginata* and *Poria cocos* to determine whether or not these fungi would induce the development of brown patch. The trees were in healthy stands of pine at Dadeville, Alabama, and Calhoun Falls, South Carolina. Each root was given two inoculations spaced 24 to 30 inches apart, one with fungus inoculum and the other with sterile cooked wheat. The root bark was wounded by cutting 6 slits about one-fourth inch long. The inoculated roots were removed for examination during the sixth and eighth months after inoculation. The amount of brown patch was calculated as the percentage of total bark surface affected within the limits of its maximum linear extent.

In both localities, the inoculations (Table 3) with *Torula marginata* resulted in brown patch, and the number of affected roots varied from 67 to 100 per cent per series. All of the isolates, except *Torula* 69, produced lesions with deep resinous cracks, sometimes extending to the wood, large pitch pockets in the bark, and pitch infiltration in the underlying wood as far as the third ring. Large masses of pitch-infiltrated soil were attached to some of the lesions associated with deep wood infections and they had the characteristics of pitch cankers. The number of lesions with pitch pockets and pitch-infiltrated wood in the series with *Torula* 50 and 56 was larger in South Carolina than in Alabama. Although the reason for this difference is not known, it is suspected that it may be a difference in soil conditions. *Torula marginata* was reisolated from brown patch on inoculated roots in both localities.

There was no evidence of brown-patch formation or infection where the roots had been inoculated with sterile wheat and in most instances the bark incisions had completely healed.

[6] Colors according to Ridgway (8).

The isolates of *Poria cocos* caused brown patch on roots in both localities (Table 3), and the percentage of affected roots varied from 50 to 100 per series. In addition to brown patch, the isolates produced deep lesions that sometimes extended into the wood, pitch pockets, and heavy exudation of pitch. Lesions resulting in deep wood infections were covered with large masses of pitch-infiltrated soil and had the characteristics of pitch cankers. There was no evidence of brown patch or infection where the roots had been inoculated with sterile wheat and most of the bark incisions had healed. *Poria cocos* was reisolated from brown patches on inoculated roots in South Carolina and Alabama.

TABLE 3.—*Pathogenicity of Torula marginata and Poria cocos on roots of healthy shortleaf pines 4 to 6 inches in diameter at breast height*

Fungi	Average root diameter	Inoculated roots[a]	Inoculated roots with brown patches[b]	Bark surface affected by brown patches[c]	Brown patches with			Inoculated roots with wood infection
					Cracks	Pitch pockets	Resinosis[d]	
	Inches	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Torula 50[e]	0.7	16	88	69	57	0	100	21
" 50*[f]	0.9	5	100	60	60	40	100	100
" 56	0.6	16	94	81	60	20	93	27
" 56*	0.8	14	100	64	36	50	100	57
" 69	0.6	3	67	45	50	0	0	0
Poria 12	0.7	15	100	82	93	20	93	67
" 12*	0.8	8	88	52	43	43	100	29
" 15	0.8	4	50	23	100	0	100	50
" 53	0.7	14	100	79	79	7	86	14
" 53*	0.8	9	67	59	33	33	100	17

[a] Each root received 2 inoculations, one with fungus and the other with sterile wheat.
[b] None of the inoculations with sterile wheat produced brown patch or infections; therefore, data were omitted in table.
[c] Calculated as per cent of bark surface within maximum linear extent of brown patch.
[d] The term "resinosis" means heavy external flow of pitch.
[e] Stock culture number of the isolate.
[f] Inoculations marked with an asterisk were made in South Carolina; the others were made in Alabama.

Torula marginata and *Poria cocos* thus are capable of not only producing brown patches but also of causing infections that extend into the wood on roots of healthy shortleaf pines growing under natural conditions.

Pathogenicity of Torula marginata and Poria cocos on Roots of Shortleaf Pine Seedlings

The possibility that the dieback of the feeding roots on little-leaf trees is caused by fungi was studied further by testing the pathogenicity of *Torula marginata* and *Poria cocos* to the roots of 2-year-old shortleaf pine seedlings. Nonsterile soil was used in this experiment. The roots of the seedlings were inoculated at the time of transplanting by scattering the grains of cooked wheat inoculum through the soil about one-half inch from the roots. The controls were inoculated with sterile cooked wheat.

All of the isolates of *Poria cocos* spread rapidly in the soil and caused heavy losses (Table 4) of plants in a relatively short time. For all isolates, the number of plants killed in 12 to 90 days varied from 79 to 100 per cent per series. In single series with isolates 53, 15, and 12, from 88 to 100 per cent of the plants were dead on the twelfth day after inoculation. The mycelium spread rapidly through the nonsterile soil and poroid mats formed on the surface of the soil in 14 days in some of the pots.

The feeding roots of the affected plants were decayed and the infections in the taproot extended upward for varying distances, and in a few instances involved the entire length. The bark on infected taproots was loose and dark brown and the stele was light brown, both being sharply demarcated

TABLE 4.—*Pathogenicity of Torula marginata and Poria cocos on roots of 2-year-old shortleaf pines grown in pots of unsterilized soil*

Total		Duration	Inoculum	Plants dead
Pots	Plants			
No.	*No.*	*Days*		*Per cent*
6	24	185	*Torula* 50[a]	79
6	24	185	*Torula* S4[b]	83
4	16	185	*Torula* 56	75
6	24	185	Sterile wheat	21
6	24	185	Sterile wheat	13
6	24	12	*Poria* 53	100
6	24	90	*Poria* 53	79
6	24	12	*Poria* 15	100
6	24	10	*Poria* 12	88
4	16	90	*Poria* 12	100
6	24	78	Sterile wheat	8
6	24	90	Sterile wheat	0

[a] Stock culture number of the isolate.

[b] P. V. Siggers isolated *Torula* S4 from roots of slash pine seedlings at a forest nursery in Mississippi.

from the live tissues. *Poria cocos* was reisolated from the steles of the infected taproots. Roots of all sizes killed by *Poria* were soon invaded by *Trichoderma*, which caused the steles to turn dark green or black.

The isolates of *Torula* (Table 4) were definitely slower in their action than those of *Poria*, and the seedling losses occurred sporadically throughout a much longer time. The seedling losses in single series of 3 different isolates were 75 to 83 per cent during 185 days. The losses in the two control series were only 13 and 21 per cent. *Torula* S4, which is one of the isolates from a Mississippi nursery, caused a slightly higher loss than the brown-patch isolates. Although the losses from *Torula* are high, they are not quite so high and did not occur so rapidly as the losses from *Poria*.

Both *Poria cocos* and *Torula marginata* were pathogenic to the roots of 2-year-old shortleaf pine seedlings.

The feeding roots of the affected plants were decayed and the deeper parts of the taproots were killed. By the end of the experiment the survivors among seedlings inoculated with *Torula* had produced much less shoot

growth than the plants in the controls, which indicated that the *Torula* may have a stunting effect on shoot growth.

A further test of the pathogenicity of *Poria cocos* and *Torula marginata* was made by inoculating the roots of 2-year-old shortleaf pines that had been potted for several months. The roots were inoculated by placing a tablespoonful of cooked wheat inoculum in the soil about one inch from each seedling. The controls were inoculated with sterile wheat.

The plants in all of the series remained healthy but dormant from the time of inoculation in November, 1942, until the last week in April, 1943. Dying of seedlings started during the last week in April, 1943, and it continued throughout May. The percentage of dead plants was high for all of the series inoculated with fungi, averaging 70 per cent for the 2 series with *Poria*, and 58 per cent for the 2 series with *Torula* (Table 5). Seedling

TABLE 5.—*Pathogenicity of Poria cocos and Torula marginata on the roots of established shortleaf pine seedlings in pots*

Inoculum	Pots	Plants inoculated	Dead plants
	No.	*No.*	*Per cent*
Sterile wheat	25	70	9
Poria 15[a]	14	45	71
Poria 53	8	20	70
Torula 50	10	32	63
Torula 56	16	45	53

[a] Stock culture number of the isolate.

losses in the control series were low, averaging only 9 per cent. Both *Poria cocos* and *Torula marginata* are pathogenic to the roots of established shortleaf pine seedlings.

The roots of 2-year-old shortleaf pines were inoculated with *Poria cocos* according to Wellman's (14) technique. The fungus was grown in a liquid medium consisting of 0.004 M KH_2PO_4, 0.008 M $Ca(NO_3)_2$, 0.004 M $MgSO_4$, and filtered extract of 200 g. of potatoes per liter. The inoculated seedlings were planted in pots of nonsterile soil. An attempt was made to determine whether or not the addition of humus to the soil would affect the pathogenicity of the fungus by potting the seedlings in one of the three series in a mixture of 2 parts of coarsely screened woods humus and 3 parts of soil, by volume. Each series consisted of 6 pots with 4 seedlings per pot.

The seedlings in each series remained healthy but dormant from the time of inoculation in November, 1942, until the last week of April, 1943. Dying of seedlings in all of the series started during the last week of April at the time of new shoot growth and continued throughout May. The total seedling losses were high in all of the series: 54 per cent for *Poria* 53 in soil; 75 per cent for *Poria* 53 in soil with added humus; 79 per cent for *Poria* 15 in soil. The addition of humus to the soil at the time of inoculation did not have an appreciable retarding effect on the pathogenicity of the fungus.

Further work was started during the summer of 1943 to test the effect of the addition of woods humus to the soil on the pathogenicity of *Poria cocos*

and *Torula marginata* to the roots of pine seedlings. Two parts of coarsely screened woods litter were mixed with 3 parts of sandy loam soil, by volume. Three-year-old shortleaf pines were planted at the time of inoculation in metal flats measuring $12 \times 24 \times 8$ inches. The roots were inoculated by mixing 2 tablespoonfuls of granular cooked wheat inoculum with the soil used for setting the roots of each seedling.

The percentages of dead plants (Table 6) resulting from inoculations with *Poria cocos* and *Torula marginata* were large in both the soil and the soil plus humus series, varying from 53 to 83 per cent per series. The percentages of dead plants in the control series were low, being 5 per cent or less for both soils. Since the losses from *Poria* were larger in the straight soil series, and the losses from *Torula* were larger in the soil plus humus series, it is concluded that the addition of humus at the time of inoculation did not definitely affect the pathogenicity of these 2 fungi.

TABLE 6.—*Effect of addition of humus to soil on pathogenicity of Poria cocos and Torula marginata to roots of shortleaf pine seedlings*

Kind of soil	Inoculum	Flats	Plants inoculated	Dead plants
		No.	*No.*	*Per cent*
Straight soil	Sterile wheat	6	60	3
do	*Torula* 56[a]	3	30	53
do	*Poria* 53	3	30	83
Soil plus humus	Sterile wheat	6	60	5
do	*Torula* 56	3	30	60
do	*Poria* 53	3	30	63

[a] Stock culture number of the isolate.

The results of the seedling inoculations have demonstrated that *Poria cocos* and *Torula marginata* are capable of destroying the fine lateral roots as well as the taproots of shortleaf pine seedlings. *Poria cocos* was definitely more virulent, as shown by the higher percentages of dead plants, than *Torula marginata* in all of the tests. The pathogenicity of the tuckahoe fungus (*Poria cocos*) confirms Wolf's (15) assumption, which was based on a detailed study of the characteristics of the tuckahoes of *Poria cocos* on pine roots, that the fungus is probably parasitic. In a later paper, Wolf (16) reported that the tuckahoe fungus on maize was a pure saprophyte, but he reiterated the fact that on pine the evidence indicates that the sclerotia are the result of pure parasitism. Weber (13) did not state whether or not the tuckahoe fungus (*Poria cocos*) was parasitic on the roots of orange trees. More recently, Davidson, Campbell, and Vaughn (5) reported that *Poria cocos* causes a brown rot of roots and trunk heartwood of oak and other hosts. The results of the present investigation indicated that the tuckahoe fungus must be regarded as a root pathogen. Although it has been demonstrated that the strain of *Poria cocos* isolated from tuckahoes associated with roots of little-leaf trees is pathogenic to the roots of pine seedlings, it is not known whether the fungus is capable of attacking in like manner the feeding roots of adult pines.

A review of the literature failed to reveal any information on the pathogenicity of species of *Torula* to the roots of pine. According to Boyce (1), *Torula ligniperda* is associated with discolorations in hardwoods, and the fungus has been found causing stain in the heartwood of living paper birch and yellow birch. Cooke (4) has reported that Ravenel found *Torula diversa* Cke. on leaves of *Agave* at Darien, Georgia. The results of the present investigation have demonstrated that the *Torula* associated with brown patches is pathogenic to the roots of pine seedlings. As in the case of *Poria,* it is not known whether the fungus is also capable of attacking the feeding roots of adult pines.

SUMMARY

A serious deterioration and dying of southern pines known as little leaf is widely distributed in the Piedmont region of several Southern States and the upper coastal plain of Alabama. Little-leaf trees have yellowed needles, shortened internodes of the twigs, and reduced diameter growth, and they die prematurely. This paper has presented the results of studies on the destruction of roots by pathogenic fungi.

No pathogenic fungi that appear to be responsible for the little-leaf disease were found associated with the aboveground parts.

The root systems of little-leaf trees are consistently more defective than those of healthy trees. The root defects that have attracted attention are dieback of feeding roots, dead primary and lateral roots, pitch cankers on the larger roots, and the excessive exfoliation of bark on the smaller roots, known as brown patch.

There was no observable difference in the gross morphology of the root systems of diseased and healthy trees. Roots of all sizes on little-leaf trees and large primary roots of healthy trees are usually covered with brown patch. On healthy trees, the roots less than one inch in diameter have only small and widely scattered brown patches.

There was no observable difference in the condition of the mycorrhizae of little-leaf and healthy trees.

Brown patches occur normally on roots of healthy trees of 4 species of southern pines. It is the abundance rather than the presence of brown patches on pine roots that is regarded as an important symptom of little leaf. A species of *Torula* is consistently associated with newly formed brown patches.

Root-bark phloem contains abundant starch over much of the year. An abundance of deep brown patches, such as occurs on roots of little-leaf trees, must result in a reduction of the food-storage capacity of the roots.

Pitch cankers occur on the roots of diseased and healthy shortleaf pines.

Sclerotia of a soil-inhabiting fungus known as *Poria cocos* were found frequently on roots of little-leaf trees but not on healthy trees.

The cultural characteristics of the strain of *Poria cocos* isolated from tuckahoes and the species of *Torula* associated with brown patches are described. The new specific name of *Torula marginata* has been given to the brown patch *Torula.*

Poria cocos and *Torula marginata* produced brown patches and wood infections when inoculated on roots of adult shortleaf pines. When inoculated on the roots of shortleaf pines 2 and 3 years old grown in pots of nonsterile soil, both fungi attacked the roots and killed a large percentage of the plants. The percentages of dead plants were higher in the *Poria* series than in the *Torula* series. The addition of woods humus to the soil at the time of inoculation failed to affect the pathogenicity of *Poria* and *Torula*.

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RESERVE FOOD STORAGE IN SHORTLEAF PINE IN RELATION TO LITTLE-LEAF DISEASE

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INTRODUCTION

The symptoms of the little-leaf disease of pine are strongly suggestive of malnutrition. Twig growth is abnormally short, the needles are short and usually yellowish, the radial growth of the trunk is greatly reduced in the later stages, and by the time the trees die the crowns are very thin. The extent to which the various organs decline as the disease progresses has been presented in detail by Buchanan (2). In studying the roots of little-leaf trees Jackson (9) found that the scaly, scabby root condition called brown patch is due to excessive and premature formation of rough bark. On diseased trees the cork cambium cuts deeply into the phloem over wide areas of small roots, resulting in the loss of much of this tissue, wherein the greater portion of the root's food reserves is stored. Since at least one and possibly two fungi appear to be associated with the brown patches, it seemed possible that little leaf was the result of root starvation owing to reduction of the food storage capacity of the roots by excessive brown patch (9). On the other hand, excessive brown patch may follow root starvation, as a result of reduced synthesis of food in the crown due to some undetermined cause. A knowledge of food storage conditions, particularly in the root, seemed necessary to an understanding of the reasons for the symptoms and behavior of the disease.

A generally low carbohydrate content throughout a little-leaf tree would indicate a systemic disturbance such as might be induced by a virus disease or unfavorable soil conditions, or possibly by the action of organisms detrimental to the absorptive system. Localization of deficient or excessive reserve foods might indicate specific disorder of certain organs. For example, White-Stevens (18) points out that several investigators have demonstrated that the foliage of boron-deficient plants has an excessive accumulation of carbohydrates whereas the roots of such plants are strikingly deficient in food reserves.

The present study involved the quantitative analysis of reserve foods in the shortleaf pine (*Pinus echinata* Mill.), from samples taken each month of the year (except one) from healthy trees in Alabama, within an area where

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little leaf is common, from diseased trees in this same area, and from healthy trees near Asheville, N. C., about 40 miles from the nearest known little-leaf disease. Since the roots of diseased trees are altered in a way that might explain the trees' decline, attention has been focused on the reserve food content of the roots, supplemented with parallel data on the trunks, throughout the course of one year, and data on food in the needles at one period in the spring.

SOME EARLIER WORK ON DISTRIBUTION OF CARBOHYDRATE RESERVES IN TREES

Food reserves in plants are of many types, consisting mainly of carbohydrates, proteins, fats, and oils. In trees the carbohydrates, largely starch, are the most prevalent. Sinnott (16) and Preston and Phillips (11) have reviewed early work dealing with the possibilities that hemicellulose[2] is also a functional food source. The role of hemicelluloses as reserve foods has not been convincingly demonstrated, and opinion prevails that if they have such a role, it may be only under extreme circumstances when starch and sugars have been exhausted. Very little of the earlier work on reserve foods was done on roots, and most of the observations were based upon qualitative iodine tests for starch, rather than upon chemical analyses.

Preston and Phillips (11) have given an excellent review of the state of knowledge of tree reserve foods in 1911. Their review and experiments indicated the following:

1. During the winter the starch content drops to a minimum in the stems and twigs of most trees in temperate climates.

2. A few species, both hardwood and softwood, show considerable increase of fat in phloem and xylem in winter, but not enough to account for the amount of starch that disappears.

3. In the roots the transformations do not keep pace with those in the stem, and starch remains the entire year, with the greatest reduction occurring in spring.

4. Fabricius (4) stated that the older stem of *Picea excelsa* does not transform its starch to so great an extent as do the younger stems. Preston and Phillips' own work also showed less change in older stems.

5. Sablon (13) maintained that the maximum for total carbohydrate reserves for deciduous trees is at the time of leaf fall in the autumn, whereas the maximum for evergreen trees is at the time of opening of buds in the spring.

6. Sablon (12) and Schellenberg (14) concluded that a principal carbohydrate reserve of trees in winter is hemicellulose, and attempts were made by the Europeans to demonstrate this both by chemical analysis and by evidence of changes in the thickness of cell walls and wall erosion, in the spring.

Sinnott (16), following the work of Fischer (5), classified a large number

[2] The term is here used as a collective one, for those polysaccharides that bridge the gap between true cellulose and the clearly recognized reserve carbohydrates like starch (7, p. 40).

of genera of trees and shrubs as "starch trees" or "fat trees," depending upon the predominant food reserve in pith and wood of twigs and young branches during mid-winter. Most of the gymnosperms were classified as fat trees, a few as starch and fat trees, and none were called starch trees, while the order was reversed for angiosperms. Since Sinnott's test for fat was simply staining with Sudan III, he must have classed as reserve fats the resins of the conifers he worked with. In fact, he mentions their accumulation in ray parenchyma and resin canal epithelium. These resins stain readily with Sudan III but generally are not regarded as food reserves, being the oxidation products of terpenes, and largely irreversible. Miller (10) states that aside from their protective influences, no biological use of these substances is known. The interpretation of Sinnott (16), Fischer (5), and others, to the effect that these resins are food reserves affects acceptance of their views on food transformations. Sablon (12) did not regard the fats as major food reserves, but maintained that when starch decreased in winter the conversion was largely to hemicellulose, and that digestion of this reserve hemicellulose was an important source of carbohydrate in the spring.

Sinnott (16) states with respect to stems, twigs, and small branches, that "in all species starch disappears in the fall almost completely from phloem and cortex, and even in the starch trees it is much reduced in the wood as well." The present work refutes this for shortleaf pine. He further states that his data show that the character of the food reserve in any cell depends primarily upon the ease with which water or substances carried by water have access to the cell. Where the movement of liquids is slow and difficult, the reserve persists as starch; where such movement is easy, starch disappears at the beginning of winter and fat is produced. He does not say whether he considers that this fat changes back to a carbohydrate again to be used by the plant in the spring. Since much of his "fat" was resin, such change apparently cannot take place.

Traub (17) found that the reserve carbohydrates in peach twigs reached their maximum just after leaf fall, and that they were made up largely of pentosans and to a much less extent of hexosans.

In reviewing past work it is necessary to keep in mind continuously the organ discussed, whether young twig, old trunk, or root, and the kind of plant—deciduous or evergreen, tall tree or small shrub. Too often authors refer to the carbohydrate content of a tree at a certain time when they really mean a specific organ such as the twig, or the root. Few workers have studied reserve food changes in all important organs of a plant simultaneously.

METHODS

From the high starch content in the outer phloem of shortleaf pine roots over much of the year, easily demonstrable with or without the use of iodine-potassium iodide (IKI) (Fig. 1, B, by Jackson (9)), it was obvious that starch is undoubtedly a very important food reserve in the root. In quantitatively studying these reserves from the standpoint of comparing little-leaf

trees with healthy trees, analysis must include at least starch, the sugars, and the carbohydrates intermediate between them. Since the question of whether hemicelluloses normally function as reserve foods is not settled, the analysis did not include hemicelluloses. Neither was an analysis of pentosans made. From the fact that in the shortleaf pine the storage tissues of wood and bark are gorged with starch over much of the year, it appeared most reasonable to use as an index of general organic nutrition the sum of starch, sugars, and their intermediate products. To determine the best method of hydrolyzing the carbohydrates to glucose, samples from a single lot of ground root bark were digested with takadiastase, digested with a proprietary diastase preparation, or were autoclaved with 3 per cent hydrochloric acid, which not only hydrolyzed the starch but the hemicellulose as well. The effect of clearing with lead acetate following hydrolysis was also tested. Table 1 gives the results of these preliminary tests, using the Shaffer-Somogyi method of sugar analysis, as described by Heinze and Murneek (8).

TABLE 1.—*Amount of reserve carbohydrate in root bark as determined by enzyme and acid hydrolysis*

Treatment[a]	Reserve food, in terms of glucose, as a percentage of oven-dry weight of bark
Takadiastase, not cleared	19.5
Proprietary enzyme compound, not cleared	14.2
Proprietary enzyme compound, cleared[b]	11.3
Acid hydrolysis, not cleared	31.2
Acid hydrolysis, cleared	17.0

[a] Eight replications of each test.
[b] Neutral lead acetate followed by potassium oxalate.

Takadiastase seemed to hydrolyze the reserve carbohydrate somewhat more completely than the other enzyme preparation, and was far less drastic than the acid hydrolysis, which took out considerable substance that might not serve as reserve food. It is felt that the hot-water-soluble carbohydrates produced by takadiastase digestion plus the other water-soluble carbohydrates come close to representing the more important food reserves of the shortleaf pine. By adopting this method for all subsequent analyses, all data are comparable and the nature of the carbohydrates determined is understood.

Each month two little-leaf trees and two nearby healthy trees were cut near Dadeville, Alabama, and two healthy trees near Asheville, North Carolina. The trees were between 6 and 8 inches in diameter, at breast height, and dominant or codominant. A 6-inch disc was cut from the trunk at 5 feet above the ground, and a 2-foot-long section from each of 2 roots about an inch in diameter was grubbed out. Preliminary tests had shown that the carbohydrate content of the trunk at 5 feet well represented the stem up to a height of 25 feet, that the bulk of the extending roots range from ½ to 1½

inches in diameter, and that within these limits root size had little effect on carbohydrate content on a dry-weight basis. These tests determined the type of sampling. The dimensions of the pieces were measured and the bark area and wood volume computed. All of the root wood and the bark from the two 2-foot root sections and the stem piece were ground in a Wiley mill. Since the stem block was larger than needed for a wood sample, two sectors of wood were cut out of each disc and their volumes computed. Immediately after collecting the samples in the field they were dried for two or three days at 75° C. to fix them. They were then ground. The sawdust was dried to constant weight at 75° C. Two 2-gram samples were taken from each batch. We thus had duplicate samples each month from two localities: two diseased and four healthy trees in all, one stem sample and two root samples from each tree, and from each sample both bark and wood tissue, separately.

The 2-gram samples were steeped in ether for three days to remove the resins; they were filtered and the residue and the filter paper returned to the flask; 100 ml. of distilled water were added to each and they were simmered for 20 minutes to saturate the sawdust; after cooling, 50 mg. of taka-diastase were added to each and the flasks kept at 38° C. for 24 hours with frequent agitation; and then they were autoclaved at 15 lb. pressure for one hour, and filtered. Ten ml. of filtrate were drawn off if bark, and 25 ml. if wood, and this made up to 50 ml. with distilled water, and 5 ml. of concentrated hydrochloric acid were added. The flasks were then simmered for 2 hours in a reflux condenser to completely hydrolyze to glucose all carbohydrates now in solution. The solutions were neutralized with sodium hydroxide solutions, first with a saturated solution, finishing with a solution 10 per cent of saturation; 3 ml. of a saturated solution of neutral lead acetate were added to clear the tannins, etc.; the solutions were filtered, each onto 9 grams of potassium oxalate to remove the lead, filtered again and made up to 150 ml. with distilled water. Heinze and Murneek's (8) description of the Shaffer-Somogyi method was followed to determine the amount of glucose in the final solution. It is of interest that, whereas Heinze and Murneek got complete reduction by their alcoholic extracts of plant tissues after 15 minutes, a 45-minute heating period was required to get complete reduction with the present aqueous extracts. In the case of wood extracts, clearing with lead acetate had no effect upon the final result, and hence was not done in most of the work.

The method is remarkably precise. Duplicate samples gave almost identical results, while the slightest difference in amounts in the tissues showed up clearly. The procedure is time-consuming, and in the present case one month's samples were completed just in time for the next, with an assistant devoting full time to the 72 samples analyzed each month.

The analyses provided figures on the total carbohydrates, from starch and the higher sugars to glucose, all converted to and expressed in terms of glucose. This fraction will be referred to as reserve food. By weighing the total dried wood and bark samples, and by getting the volume of the wood

samples and the area of the bark samples, it was possible to express the reserve food in the following ways: for both bark and wood as a percentage of the total oven-dry weight, in the case of bark in terms of milligrams of glucose per square inch of bark, and in the case of wood in terms of milligrams of glucose per cubic inch of wood. For bark, expressing reserve food as a percentage of the oven-dry weight has disadvantages. Shortleaf pine trunks vary considerably in the thickness of the outer bark. Since the food reserves are largely confined to the living cortical tissue, if one tree had thick outer bark, its food content, expressed in terms of percentage of the oven-dry weight of the bark would be very much lower than another tree with thin outer bark, but the same amount of inner bark. For trees of 6 to 8 inches' diameter, the bark thickness often varied an inch or more between trees. To minimize the influence of the outer bark on the final figures, the stem sections were peeled close to the inner bark before stripping the bark for grinding and weighing. The root bark was not trimmed or scraped, but only the dirt was washed off, before peeling and grinding.

RESULTS

The great bulk of the bark starch is in the outer phloem. There is also abundant starch in the ray parenchyma, and in those phloem parenchyma cells, scattered among the sieve tubes, which will later expand to form the major phloem storage tissue. Starch is regularly absent from the sieve tubes and from the cortical cells cut off by the phellogen and constituting the outer bark. These patches of outer bark on small roots are referred to as brown patch, since they give the impression of being dead lesions on the normally reddish turgid bark of these roots. In the wood, starch occurs in the living elements such as ray parenchyma, pith, and resin canal epithelium.

The Annual Cycle of Reserve Foods in Trunk and Root

Figure 1 presents the carbohydrate cycle over the course of one year for healthy and little-leaf trees. This figure is put on a basis of milligrams of glucose (all starch and sugars being converted to glucose) per square inch of bark and per cubic inch of wood. These have proven to be better bases on which to follow the cycles than is the percentage of the oven-dry weight, since the curves are more regular. Table 2 presents the ranges covered by figure 1 in terms of percentage of the oven-dry weight.

The following conclusions may be drawn from figure 1:

1. There was good agreement between the cycles for healthy trees from North Carolina and Alabama. The sudden dip of the June point for Alabama root bark is unexplained. The North Carolina curves, based on trees far from the little-leaf belt, were more regular than the Alabama curves, based on trees intermixed with little-leaf trees.

2. Roots of little-leaf trees had much less reserve food than roots of healthy trees, and their graph shows no cyclical pattern because the great variation between diseased trees overshadows the cyclical changes.

3. The stem and roots of normal trees had their maximum accumulation of reserve food in the early spring. This checks with Sablon's (13) observations on *Pinus australis* Michx. f. Minimum reserves occurred in the roots in the fall, and in the stem the minimum was maintained all year except for a short high period in the spring.

4. Little-leaf root bark samples taken in February, May, and July were almost completely depleted of food. Although tests showed between 60 and 75 milligrams of glucose per square inch (about 8 per cent), inert outer bark will test over 4 per cent because of substances that, although not associated with living tissue, are converted to glucose in the process of extraction.

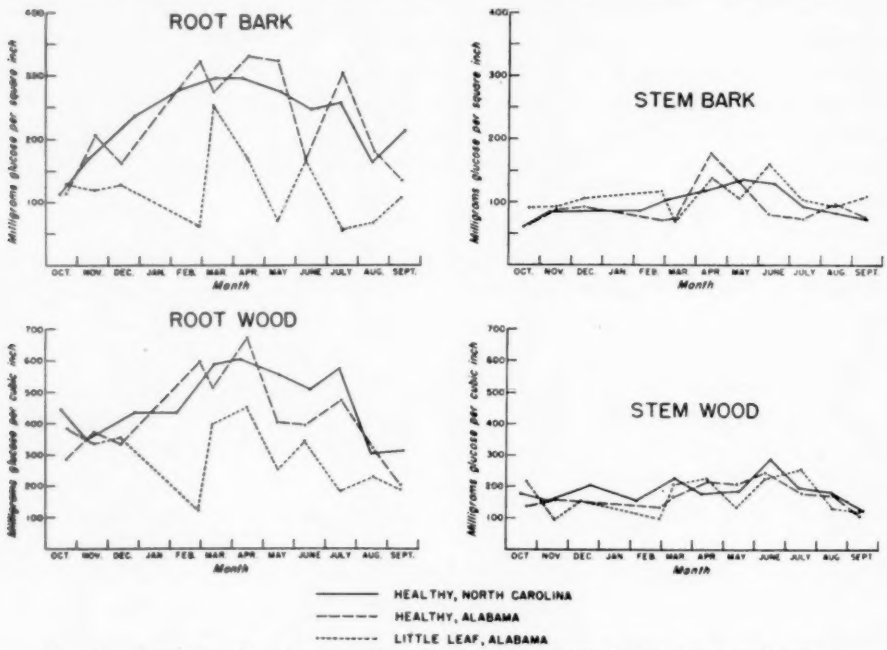

FIG. 1. Annual cycle of reserve food changes in shortleaf pine stems and roots.

Duplicate runs from the same batch of sawdust gave almost identical results. Variation in carbohydrate content between healthy trees or between roots of the same healthy tree, for a given month, was usually not great. The variability between roots of the same tree, expressed as a percentage of the mean for the two roots of each tree at each month, averaged 26 per cent, and when expressed as an average did not differ between diseased and healthy trees. There was more pronounced variability, however, between the roots of certain diseased trees. In October, healthy roots were near food exhaustion, but certain little-leaf roots were extremely low at times of the year when normal roots were at their highest. In March, when normal roots had maximum food, one root of a little-leaf tree had 24 per cent reserve carbohydrates, while the other sampled root of this tree had 6 per cent. Such differences indicate that diseased trees may succumb root by

TABLE 2.—*Reserve carbohydrate as a percentage of the oven-dry weight*

Month	North Carolina				Alabama							
	Healthy				Healthy				Little leaf			
	Root bark	Root wood	Stem bark	Stem wood	Root bark	Root wood	Stem bark	Stem wood	Root bark	Root wood	Stem bark	Stem wood
	Per cent of oven-dry wt.				Per cent of oven-dry wt.				Per cent of oven-dry wt.			
October	12.1	7.2	6.7	2.0	18.1	5.3	7.8	1.6	15.6	5.5	10.8	2.4
November	21.6	5.7	8.9	2.3	25.9	7.6	11.2	1.4	15.5	5.5	10.3	1.0
December	21.0	8.4	11.6	2.5	16.2	4.8	10.4	1.4	13.6	5.8	12.8	1.4
February	28.0	7.0	12.3	1.9	31.9	9.8	9.5	1.3	8.5	2.6	9.5	1.0
March	24.2	10.3	12.4	2.4	31.0	8.7	15.0	1.5	21.6	6.0	12.3	1.8
April	30.0	11.4	12.8	1.9	33.2	9.0	23.4	2.0	18.0	6.8	14.3	2.1
May	28.0	10.1	12.0	2.2	30.6	7.6	18.1	2.2	11.0	4.0	15.6	1.4
June	26.9	8.2	12.2	3.2	19.4	7.4	11.3	2.4	15.7	6.4	14.6	2.4
July	25.7	10.1	11.0	2.3	28.1	9.0	9.4	1.8	9.0	3.4	11.3	2.4
August	17.7	5.7	10.4	2.1	20.4	5.8	11.6	1.9	8.8	4.1	10.1	1.4
September	24.2	6.0	9.7	1.3	16.0	3.5	11.2	1.1	11.8	3.8	12.4	1.2
Average	23.6	8.2	10.9	2.2	24.6	7.1	12.6	1.7	13.6	4.9	12.2	1.7

root, rather than by uniform decline of the root system. If this is so, there exists the implication of pathogenic action rather than unfavorable soil conditions. The variability between the two trees cut at each period averaged 27 per cent for healthy trees and 64 per cent for diseased trees, and this difference was highly significant, statistically. This high variability from tree to tree for diseased trees was due to unequal severity of the disease, and accounts for the irregularity of the cyclical graph for diseased trees in figure 1.

5. The seasonal effects in root bark and wood were similar and were more pronounced than in the stem. Seasonal effects for stem bark and wood were similar to each other, were more irregular than for corresponding root tissues, and the general level was lower.

The importance of keeping in mind the plant and plant organ in question can be illustrated by a few examples. Aldous (1) found that in the stems of sumac (*Rhus glabra* L.) and buckbrush (*Symphoricarpos orbiculatus* Moench) the least starch appeared while the plants were in flower and the maximum of starch was regained by about August 15. The shortleaf pine stem and root in the present work (Fig. 1) approached a minimum of food reserves in mid-August. Traub (17) found that the hexosans disappeared almost entirely from the cortex of apple twigs during the winter, and most observers have noted a depletion of starch in winter in stems and twigs of deciduous plants. In the shortleaf pine, however, the food reserves of the stem held constant all winter, whereas the root reserves, mostly starch, increased steadily and markedly all winter, reaching a peak in early spring (Fig. 1).

Reserve Food Content of Stem, Root, and Foliage

Having in mind the profound effect of season on carbohydrate content of roots, we can now consider the average amount of stored food for the different tissues for the year as a whole. Figure 2 presents these averages for stem and root, and is based upon the data that were used to construct the seasonal curves.

The roots of healthy trees, both bark and wood, had roughly twice as much food per unit area or volume, as roots of little-leaf trees. The roots of little-leaf trees at times were almost completely devoid of food.

The stems of healthy trees, both bark and wood, had no more food than the stems of diseased trees on either unit basis used.

In healthy trees, root bark had about twice as much food as stem bark, on either unit weight or unit area basis. Root bark had about three times as much food as root wood, and seven times as much as stem wood, on a unit-weight basis. In the average normal root 1 inch in diameter, 68 per cent of the reserve food is in the bark. The root bark is thus a tissue of great storage capacity.

In interpreting the bark data in figures 1 and 2, and table 2, consideration must be given to the fact that a fraction of the carbohydrates extracted and converted to glucose in the analysis was probably not reserve food. As

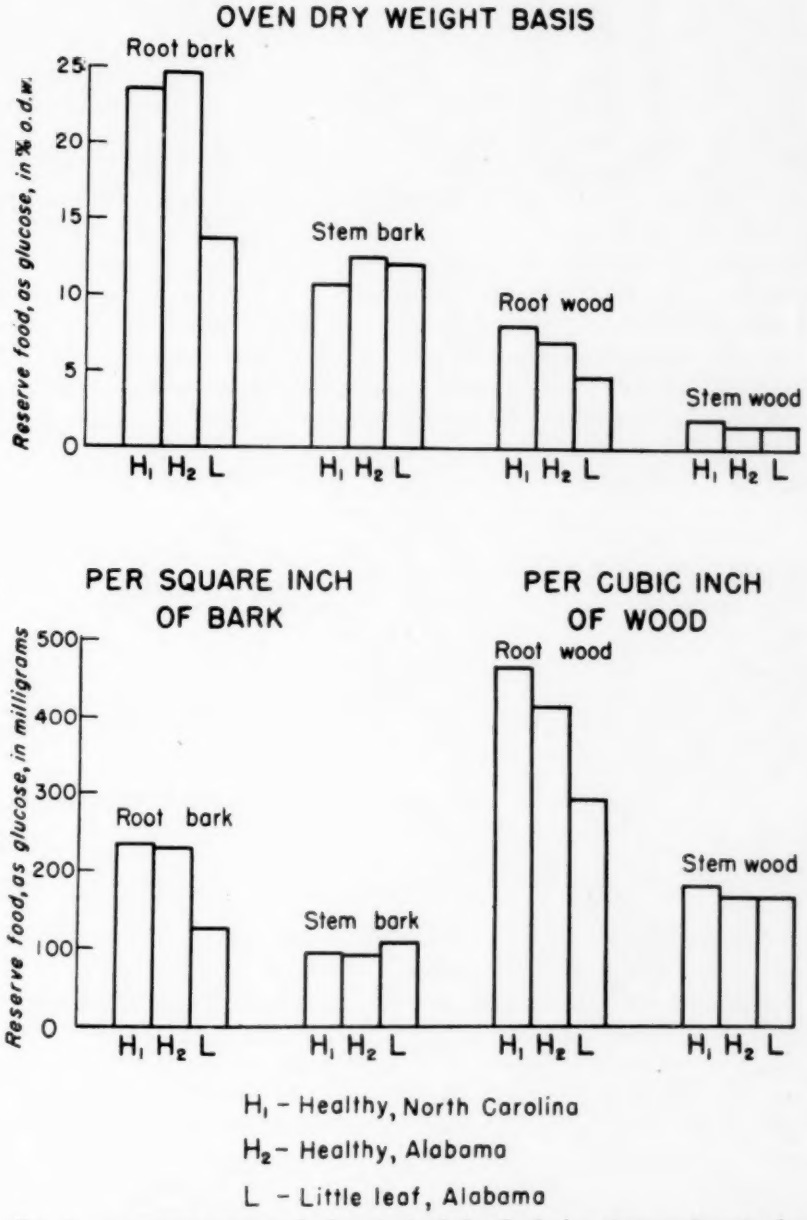

FIG. 2. A, average reserve food content of shortleaf pine stems and roots, for the year, as a percentage of the oven-dry weight; B, converted to milligrams of glucose per square inch of bark and per cubic inch of wood.

already mentioned, functionless outer bark, even when cleared of tannins and other coloring materials, tested about 4 per cent glucose. If we correct for this 4 per cent unavailable carbohydrate, in the bark histograms of figure 2, A, we find that root bark has nearer two and one-half times the reserve food of stem bark over the year as a whole, per unit weight.

Conditions did not permit so exhaustive a study of the carbohydrate content of other organs as was given the root and stem. In April, 1943, however, foliage and roots were analyzed from two healthy trees and two trees in advanced little leaf, near Pickens, South Carolina, to determine the relation of the amount of elaborated food in the foliage to the amount in the roots, between and within little-leaf and healthy trees. The investigation of this phase was also stimulated by the findings of Haas (6), White-Stevens (18), and others, to the effect that boron deficiency induced a high carbohydrate content in foliage and a low content in the roots. Some indications of benefit to little-leaf trees by additions of boron made this of particular interest.

The root bark of the two healthy South Carolina trees had 303 mg. of reserve food per square inch (33 per cent on oven-dry weight basis), comparing very favorably with healthy North Carolina (30 per cent) and Ala-

TABLE 3.—*The reserve food content of needles and root bark of little-leaf and healthy trees in April, 1943*

Carbohydrate content of tissues	Healthy trees	Little-leaf trees	Percentage reduction
Leaves			
Carbohydrates, as per cent of oven-dry weight	16.6	11.5	31
Carbohydrates, as per cent of fresh weight	7.3	4.7	36
Carbohydrates, as mg. per linear inch	1.01	0.62	39
Carbohydrates, as mg. per needle	2.69	1.10	59
Root bark			
Carbohydrates, as per cent of oven-dry weight	32.9	11.8	64
Carbohydrates, as mg. per square inch	303.0	101.0	67

bama (33 per cent) trees for April of the preceding year (Fig. 1). The two diseased trees had an average of 101 mg. per square inch (12 per cent). From each of these trees 1,000 needles were removed by stripping several random twigs completely. The fascicles were removed, and the needles were measured and their fresh and oven-dry weights (at 75° C.) computed. Two-gram samples were used as in the other analyses. Table 3 presents the needle and root data for these trees.

The portion of the healthy twigs that bore needles (usually 2 years' growth) averaged, in length 4.4 times the needle-bearing twig length of the diseased trees. Buchanan (2) also found, for needle-bearing twig length, a ratio of about 4 to 1 for healthy compared with advanced little leaf. Table 3 shows that the average needle of a little-leaf tree contained only 41 per cent as much food as that of a healthy tree. Since there are only about one-fourth as many needles on the living twigs of diseased trees, the total foliage food content of the diseased trees is only about 10 per cent of normal. Since trees in advanced little leaf usually have 30 or 40 per cent of their twigs dead, we finally arrive at a food content of the green parts of trees in advanced little leaf of 3 or 4 per cent that of healthy trees. From this very great reduction in produced food we find good reason for the extremely nar-

row annual rings formed by trees in the advanced stage of little leaf, for the very low food content of the roots, at times reaching almost complete exhaustion, and for the other symptoms of decline discussed by Buchanan (2).

An extra analysis of carbohydrates in needles 1 and 2 years old on healthy trees showed the 2-year-old needles to have 95 per cent as much as the 1-year-old. Although 2-year-old needles had about as much total carbohydrates as the current year's needles, in April, there still could be a question as to whether they synthesize food at the same rate as the current year's needles.

DISCUSSION

The carbohydrate cycle for roots and stems of normal shortleaf pine, as brought out in figure 1, is very different from the cycle for hardwoods, as proposed by various authors already discussed. The varying conclusions of other authors have depended to a large extent not only upon the type of plant and the organ involved, but also upon which substances they considered reserve foods. Certainly the cycle of food reserves would be quite different depending upon whether one included, in addition to starch and sugar, in the total reserves, hemicellulose, fats, pentosans, or other substances. In the present work the amount of substances hydrolyzed by taka-diastase varied directly with the vigor of the trees, and it would thus appear that the amount of these substances (largely starch) comprises a good index of the amount of available food.

The present work substantiates certain work of Sablon (12) on deciduous trees, concerning which he states that stems have less reserves than roots, and that stem reserves vary much less than root reserves, usually having a lower maximum and a higher minimum than root reserves.

Buchanan (2) has described in detail the slow decline of the aerial parts of little-leaf trees, and Siggers and Doak (15) and Jackson (9) have described deteriorated roots, with dead root ends and excessive exfoliation of vital cortical tissue. The present work demonstrates that a marked reduction in carbohydrate synthesis accompanies the external symptoms of little leaf, to the extent that the reserve food production of the foliage as a whole of trees in advanced little leaf, is less than 10 per cent of normal. Since no evidence has been found of any pathogenic organisms in the aerial parts of diseased trees (9), it seems very likely that the disease either originates below the ground line as a pathogenic or physiogenic disturbance, or that it is a virus disease.

That the roots of diseased trees should be deficient in food whereas the trunks have essentially normal amounts suggests that, under conditions of insufficient food production, either the trunk retains its requirements at the expense of the roots, or initial root deficiency is due to excessive brown patch destroying the capacity of the root to store food. The latter theory seems less likely because some of the little-leaf roots analyzed had very little brown patch and yet were very deficient in food. Starvation of roots would be expected to result in the cork cambium cutting deeply into the phloem food

storage region. We may therefore regard the excessive exfoliation of cortical tissue in little-leaf roots as a result of the disease, and thus as a symptom, rather than as a cause. The loss of this vital tissue, however, means that if recovery from little leaf could be effected, there would, for a few years, be inadequate storage tissue in the roots to accommodate normal amounts of starch. Rapid improvement in the appearance of the trees, therefore, could hardly be expected until sufficient new root phloem was formed, even where successful treatments were in operation.

Curtis (3) has concluded, from extensive experiments and review, that carbohydrates translocated to the trunk or root are used close to where they are stored, and do not come up again to be used in shoot growth. If we accept this view, then the low food reserves of little-leaf tree roots will be reflected largely by the roots themselves. The dead root ends common on such roots, as described by Jackson (9) and Siggers and Doak (15), may be the result of this root starvation, or the death of the fine roots may be a pathogenic condition that initiates little leaf. The many possible causal factors of the abnormality are being investigated by other workers.

SUMMARY

Quantitative analyses were made of food reserves in the bark and wood of normal shortleaf pines and of trees with little-leaf disease. The analyses were made throughout one year for roots and trunks and at one period in the spring for foliage.

The roots of normal trees had their minimum of reserve food in the fall. The amount increased all winter, reaching a maximum that was approximately three times the minimum, in early spring.

The stems of normal trees reached a maximum between April and June, and maintained a more or less constant lower level from July to March.

In normal trees root bark had an average for the year of about twice as much reserve food as stem bark, on either unit weight or unit area basis, about three times as much as root wood, and seven times as much as stem wood, on a unit dry-weight basis.

In a section of normal root 1 inch in diameter, 68 per cent of the reserves are in the bark, on an average basis for the year.

The root bark of little-leaf trees had an average of less than half as much food as that of healthy trees, and at times reserve food was almost entirely absent from some roots of diseased trees. The stems of little-leaf trees had as much food as normal trees at all times.

The average needle of little-leaf trees cut in April contained only 41 per cent as much elaborated food as the average needle of adjacent healthy trees. The total foliated parts of these trees were computed to have a reserve food content less than 10 per cent of normal. Two roots from each of the same trees used in the foliage analyses showed the diseased to have a reserve food content of only 33 per cent of normal.

The symptoms of decline in little-leaf trees appear to be due to a low synthesis of carbohydrates. The reason for the low synthesis is yet to be

discovered. The roots, which normally store much more food than the stems on a unit weight basis, and which fluctuate more in amount, seasonally, suffer most from inadequate nutrition. A striking effect of this root starvation is accentuated formation of rough bark owing to the cork cambium cutting deeply into the food storage region of the phloem. Thus less storage tissue remains in the root bark, and less food has to be accommodated due to reduced synthesis. The excessive storage tissue is apparently cut off by cork cambium as it deteriorates as a result of starvation.

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FURTHER TESTING OF COPPER FUNGICIDES FOR CONTROL OF TOMATO BLIGHT IN SOUTHWEST VIRGINIA[1]

R. G. HENDERSON[2]

(Accepted for publication September 20, 1944)

Experiments by the author (2) in southwest Virginia during 1940 to 1942 inclusive showed that tribasic copper sulphate and cuprous oxide, applied either as a spray or as a dust, effectively controlled early blight (*Alternaria solani* (E. & M.) Jones and Grout), late blight (*Phytophthora infestans* (Mont.) de By.), and leaf spot (*Septoria lycopersici* Speg.) of tomato, and thereby brought about a considerable increase in the yield of marketable fruits. Dusts were about as effective as sprays in controlling these diseases. These results, together with the opinion that dusts are more practicable to use, because they are easier to apply, led to more precise comparison of effectiveness of cuprous oxide and tribasic copper sulphate applied as dusts. Because McNew (5) indicated that increases in yield resulting from fungicidal applications were much larger on plots receiving well-balanced fertilization, the experiment included tests with side applications of nitrate of soda. The two fungicides were compared mainly under field conditions, but there were supplemental studies in greenhouse and laboratory.

FIELD STUDIES

The objective of the field studies was twofold: (1) to determine more precisely the relative efficacy of cuprous oxide and tribasic copper sulphate dusts in controlling tomato diseases, and (2) to determine whether the benefit from the use of these materials as expressed in yield of marketable fruit could be further increased by supplemental applications of nitrate of soda during the growing season.

Materials and Methods

Tomato seedlings of the varieties Rutgers, Marglobe, and Pritchard grown in the greenhouse were transplanted on May 28 into the field where tests had been made the previous year (2). An application of 500 pounds per acre of 2–12–8 fertilizer was made in bands shortly before transplanting. In addition, half of the plants in each fungicidal treatment plot received supplementary applications of sodium nitrate at the rate of 100 pounds per acre on July 7, 50 pounds on July 20, and 100 pounds on August 3.

The fungicidal treatments applied to each variety, in four replicates,

[1] Paper No. 122 from the Section of Botany and Plant Pathology, Biology Department, Virginia Agricultural Experiment Station, Blacksburg, Va. This is a portion of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[2] The author wishes to acknowledge his indebtedness to Dr. Boyd Harshbarger for his assistance in making the statistical analysis of the data and to Dr. S. A. Wingard and Dr. G. C. Kent for their counsel during the investigation and assistance in preparation of the manuscript.

were: tribasic copper sulphate dust,[3] yellow cuprous oxide dust,[4] and an untreated control. The treatments were applied by a motor-driven duster on July 14, 22, 30, and August 10, 14, 22, at the rate of 40 to 50 pounds per acre. Each plot consisted of 1/60 of an acre which was further divided in order to accommodate the fertilizer treatments; so yield records were taken on plots of 1/121 of an acre containing 32 plants. The fruits were harvested as they ripened (when yellow-red), and then were graded and weighed as described previously (2).

There was considerable rainfall during the 1943 season. A heavy rain of 4.86 inches within 48 hours after the first nitrate application probably

TABLE 1.—*Summarized yields of tomatoes in 1943 from plots of approximately 1/30 acre*

Treatment	Grade	Rutgers		Pritchard		Marglobe		Av. of 3 varieties	
		No nitrate	Nitrate	No nitrate	Nitrate	No nitrate	Nitrate	No nitrate	Nitrate
		Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Tribasic dust	No. 1	328.5	280.5	242.3	243.8	248.8	212.5	273.2	245.6
	No. 2	265.5	266.3	278.8	275.0	287.3	260.0	277.2	267.1
	Culls	250.5	279.0	368.5	323.5	262.3	273.8	293.8	291.9
	Rots	24.5	40.3	22.5	26.0	27.5	28.5	24.8	31.6
Cuprous oxide dust	No. 1	388.3	304.0	307.5	305.3	334.0	261.5	343.3	290.3
	No. 2	243.3	236.5	343.8	293.3	283.8	275.3	290.3	268.3
	Culls	219.3	237.0	314.5	325.8	240.5	235.8	258.1	266.2
	Rots	71.5	58.5	39.0	30.5	43.3	39.3	51.3	42.8
Check	No. 1	264.0	237.0	187.5	193.3	190.0	178.0	213.8	202.8
	No. 2	219.5	217.5	210.8	204.3	251.0	210.0	227.4	210.6
	Culls	271.0	275.5	310.0	312.8	308.5	312.5	296.5	300.3
	Rots	42.8	35.0	25.5	22.0	29.5	33.3	32.6	30.1
Average of all treatments	No. 1	326.9	273.8	245.8	247.5	257.6	217.3	276.8	246.2
	No. 2	242.8	240.1	277.8	257.5	274.4	248.4	265.0	248.7
	Culls	246.9	263.8	331.0	320.7	270.4	274.0	282.8	286.2
	Rots	46.3	44.6	28.8	26.2	33.4	33.7	36.2	34.8

leached most of the fertilizer from the soil. The total rainfall of 6.76 inches in June and 6.58 inches in July associated with high temperatures (5.5 degrees above normal in June and 0.5 degrees above in July) created conditions favorable to the development of the pathogens. Under such favorable conditions the pathogens developed abundantly on the lower leaves of the plants during June and by early July many leaves on the upper parts of the plants were infected. The lower leaves of plants on the experimental plots were practically all destroyed by July 14, the date of the first fungicidal application. The weather remained favorable for disease development until after the first fruits were picked on August 11. After this date rainfall was

[3] A commercial dust mixture prepared by a local distributor from tribasic copper sulphate manufactured by the Tennessee Copper Company. The metallic copper content was 6 per cent.

[4] A commercial dust mixture prepared by a local distributor from yellow cuprous oxide manufactured by Rohm and Haas Company. The metallic copper content was 4.1 per cent.

extremely deficient throughout the remainder of the season and the mean temperature for August was 2.6 degrees above normal. The hot, dry weather during August probably hastened abscission of the diseased leaves. It can be safely stated that the 1943 season in the vicinity of Blacksburg was extremely favorable for the development of the tomato pathogens and relatively unfavorable for plant growth.

Results of Field Test

The yields of ripe fruits from the various plots are in table 1 and presented graphically in figure 1. An analysis of variance on the yields of

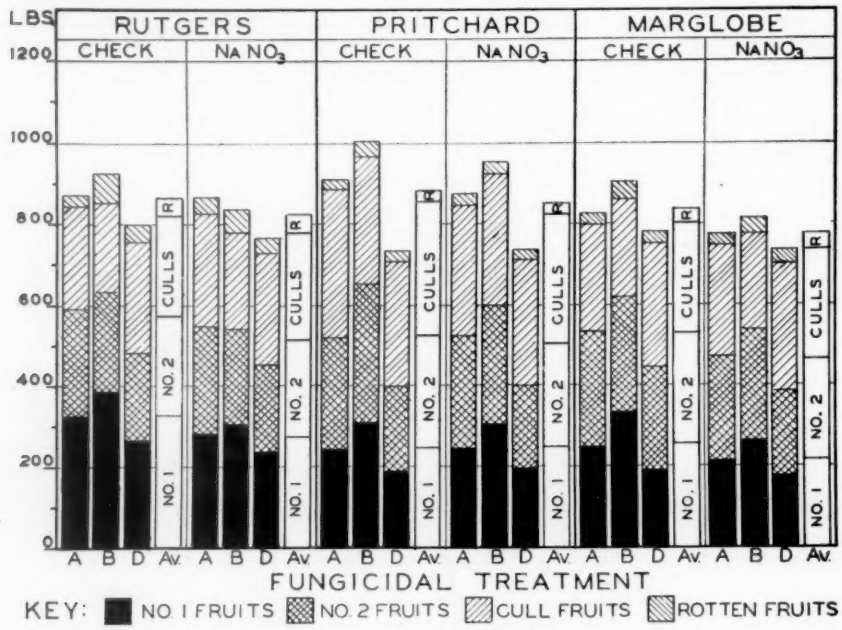

FIG. 1. Comparison of yields of fruit from the series of plots that received a basic application of fertilizer (check) with those from the series that received nitrate of soda in addition to the basic application. The unshaded column (Av.) represents the average yield for the series. The fungicidal treatments are: (A) tribasic copper sulphate dust; (B) cuprous oxide dust; (D) no treatment.

marketable fruit (No. 1's and No. 2's combined) is summarized in table 2. The average yield of the Rutgers variety was slightly higher than either of the others, but the difference between varieties was not significant. There was no difference in the response (interaction) of the varieties to the dust treatments. The average yield of marketable fruit for the three varieties from the plots dusted with cuprous oxide was 597 pounds, for tribasic copper sulphate 532, and for the undusted check 428 pounds. The differences between these yields are highly significant; that is, the cuprous oxide treatment gave better results than the tribasic copper sulphate treatment and the tribasic copper sulphate treatment, in turn, gave better results than no treatment.

TABLE 2.—*Summary of analysis of variance on pounds of marketable fruits in 1943.*

Comparison	Degrees of freedom	Sum of squares	Mean squares
Blocks	3	11,328.94	
Varieties	2	1,458.25	729.13
Error (a)	6	2,253.97	375.66
Nitrogen levels	1	2,473.38	2,473.38*
Nitrogen × Varieties	2	457.53	228.77
Error (b)	9	2,740.76	304.53
Dusts:			
Cu_2O vs. Tribasic	1	3,152.52	3,152.52*
Dusts vs. Check	1	18,564.06	18,564.06*
Dusts × Varieties	4	1,799.42	449.86
Dusts × Nitrogen	2	441.20	220.60
Dusts × Nitrogen × Varieties	4	60.14	15.04
Error (c)	36	7,483.33	207.87
Total	71	52,213.50	

* Significant at 1 per cent level.

The plots that received only the basic application of fertilizer gave significantly higher yields than those that received both the basic application of fertilizer and the supplementary applications of nitrate of soda; the average yields (three varieties) of marketable fruit from the two treatments being 542 pounds and 495 pounds, respectively. The first four pickings from the plots that received nitrate of soda were larger than those from the plots that received no nitrate of soda, but all the later pickings were smaller. The comparative picking yields from the Pritchard variety are illustrated

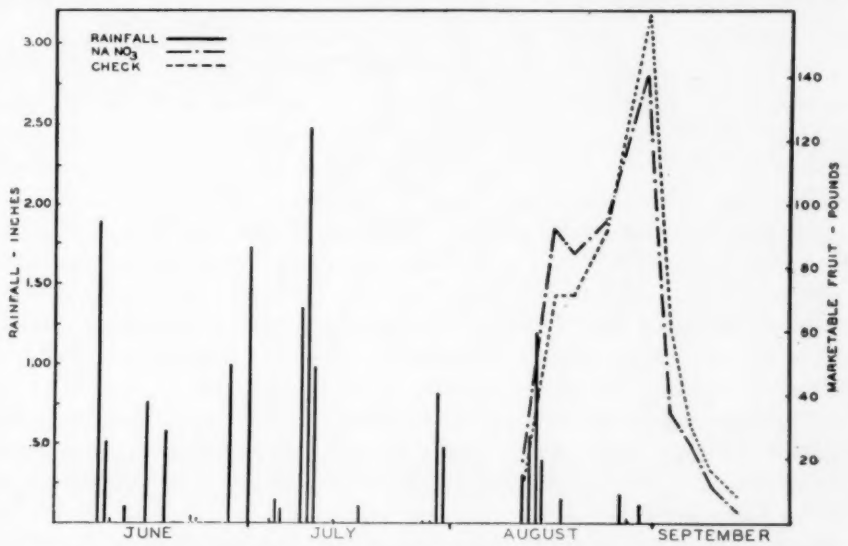

FIG. 2. Yield of marketable fruit by pickings from the Pritchard variety from 1/30 acre plots that received a basic application of fertilizer (check) as compared to the yield from plots that received nitrate of soda in addition to the basic application, and the relation of these to rainfall. The rainfall data are from the U. S. Weather Bureau records for Blacksburg, Virginia.

in figure 2. There was no interaction between varieties and nitrate fertilization, although the difference in favor of no nitrate fertilization in the Pritchard variety was much less than that in the other varieties.

The uneven distribution of rainfall in July and August (Fig. 2) probably caused the plots side-dressed with nitrate of soda to yield less than those not side-dressed. During early August when rainfall was sufficient, the pickings of fruit from the plots side-dressed with nitrate of soda were greater than those from the plots not side-dressed, but with the onset of the drought in late August the yields from these plots became less than those from plots not side-dressed. This depression of yield by the nitrate of soda during the dry period is probably explained by Weaver and Clements' work (7) in which they found that plants side-dressed with nitrate of soda developed a shallow root system and for that reason were injured more severely by drought than plants not side-dressed with nitrate of soda.

LABORATORY AND GREENHOUSE TESTS

The results of the field test indicated that the commercial cuprous oxide dust was more toxic to the tomato foliage pathogens than tribasic copper sulphate dust as judged on the basis of increased yield of marketable fruit. But since factors other than toxicity may have influenced the field results, certain laboratory and greenhouse studies were conducted to more accurately determine the relative fungicidal efficiency of the two materials. In the laboratory, the dusts were compared on the basis of their toxicity to spores of *Alternaria solani;* and in the greenhouse, they were compared on the basis of their effectiveness in preventing infection of young tomato plants by *A. solani.*

Materials and Methods

The slide test technique outlined by the Committee on Standardization of Fungicidal Tests, American Phytopathological Society (1) for testing the toxicity of fungicidal dusts was used. The test fungus was a non-chromogenic, pathogenic, sporulating strain[5] of *Alternaria solani.*

In the greenhouse tests, tomato plants of the Bonny Best variety, as recommended by McCallan and Wellman (4), were grown in a fertile potting soil in 4-inch pots and were about 8 to 10 inches high at the time the dusts were applied. Uniform lots of 5 plants each were selected. Each lot was then removed to a room adjoining the greenhouse and dusted individually.

The fungicides were applied with a plunger-type duster. Care was taken to cover the upper and lower surfaces of the leaves as uniformly as possible, but no specific quantity of dust was applied. The foliage was dry at the time it was dusted and the amount of dust that adhered to the leaves was relatively small. The plants were jarred lightly after dusting to dislodge the excess dust that fell on the more exposed leaves. After the plants were

[5] The culture of this strain of *Alternaria solani* was supplied by Dr. E. K. Vaughan, who had obtained it from Dr. W. D. Moore. It was the same strain as that described by Thomas (6).

dusted, they were placed in a moist chamber for a few hours before the spore suspension of *Alternaria solani* was applied.

The moist chamber was a glass-enclosed box large enough to accommodate about 30 pots. The floor of the box was covered with wet sphagnum moss. During the day the moist chamber was protected from direct sunlight to avoid overheating. The temperature in the chamber ranged between 21° and 28° C.

The inoculum was sprayed onto the upper and lower surfaces of the leaves by means of a hand-operated atomizer. A sufficient amount of the suspension was applied to wet the surface of the leaves but not enough to run. After being atomized, the plants were returned to the moist chamber where a high relative humidity was maintained for 36 hours.

Early blight lesions were visible on the leaves of inoculated plants in about three days but the readings were not made until after the sixth or seventh day. The fourth, fifth, sixth, and seventh leaves from the base were removed from the plant and all the lesions on all the leaflets counted and recorded as the total number of lesions per plant.

Five greenhouse tests were run. In the first four, the full strength dust as received in the package was used, but in the fifth test the dusts in addition to being used full strength were diluted with talc in the ratio of one to three, one to one, and three to one. The checks consisted of a series of undusted plants and a series dusted with talc.

Results of Laboratory Tests

The laboratory data were inconclusive because of the wide variability of results from the different tests. This variability was apparently due to the cuprous oxide being difficult to wet with water, so that a portion of the toxicant floated on the surface of the drop of spore suspension, and also to the non-uniformity of samples resulting from treatment with poorly mixed tribasic copper sulphate dust. In small dosages, cuprous oxide dust appeared to be more toxic to spore germination than similar dosages of tribasic copper sulphate dust, but in large dosages, the results were inconsistent.

Results of Greenhouse Tests

The tribasic copper sulphate dust applied to tomato plants in the greenhouse reduced the number of infections of *Alternaria solani* from an average of 120.25 per plant, as shown by the check, to 7.05 per plant; and cuprous oxide reduced the number to 1.85 per plant (Table 3). In four trials cuprous oxide consistently gave better control than tribasic copper sulphate.

Dilutions of re-mixed tribasic copper sulphate dust with talc gave progressively less control of early blight as the dilution increased (Table 4). The one-fourth strength dust gave an average of 21.75 lesions per plant, while the one-half and three-fourths strengths gave 18.25 and 14.50 lesions, respectively. Dilution of cuprous oxide dust with talc did not give a proportionate reduction in control. The one-fourth strength gave 13.25 lesions per plant; the one-half strength, 9.25; and the three-fourths strength, 10.75.

TABLE 3.—*Number of lesions developed on dusted and undusted tomato plants inoculated with Alternaria solani in 4 experiments in the greenhouse*

Treatment	No. lesions on each of 5 plants					Average per plant
	1	2	3	4	5	
Check, not dusted	104	67	83	115	96	93.00
	128	27	118	56	90	83.80
	42	29	60	75	102	61.60
	241	206	467	66	233	242.60
Average	128.75	82.26	182.00	78.00	130.25	120.25
Tribasic dust	16	6	19	1	11	10.60
	6	7	3	3	1	4.00
	3	6	14	5	1	5.80
	4	10	5	2	18	7.80
Average	7.25	7.25	10.25	2.75	7.75	7.05
Cuprous oxide dust	3	2	5	2	0	2.40
	0	1	1	3	1	1.20
	1	5	0	3	0	1.80
	4	6	0	0	0	2.00
Average	2.00	3.50	1.50	2.00	0.25	1.85

DISCUSSION

Records for the past several years show that foliage diseases on unprotected tomatoes have reduced yields 30 to 40 per cent each year in southwest Virginia. Such consistent losses indicate that spraying or dusting of tomatoes in this area would be profitable each year. However, the returns from applying a fungicide depend not only on disease control but also on seasonal factors and soil fertility which determine the growth and productivity of the crop. This was clearly demonstrated in 1943 when the environmental factors of the field test were unfavorable for optimum plant growth. The basic application of fertilizer was moderately light and a number of heavy rains

TABLE 4.—*Number of early blight lesions developed on tomato plants dusted with different dilutions of fungicides*

Treatment	Ratio dust : talc	No. lesions on each of 4 plants				Average per plant
		1	2	3	4	
Check	.....	202	168	71	311	188.00
Talc	.....	52	30	39	29	37.50
Tribasic dust	1 : 3	19	24	19	25	21.75
	1 : 1	11	12	36	14	18.25
	3 : 1	6	24	24	4	14.50
	1 : 0	10	15	2	1	7.00
Average	.....	11.50	18.75	20.25	11.00	15.38
Cuprous oxide dust	1 : 3	5	13	16	19	13.25
	1 : 1	11	16	9	1	9.25
	3 : 1	6	17	17	3	10.75
	1 : 0	5	11	4	3	5.75
Average	.....	6.75	14.25	11.50	6.50	9.75

in June and July further depleted the soil fertility through leaching of the soluble nutrients. Also, drought conditions in August and September were very injurious to the crop. Under these conditions, the increase in yield as a result of dusting was not so large as that obtained in previous years (2), but even under such adverse conditions the fruit from the dusted plants was of superior quality and there was an increase in yield of 2 or 3 tons per acre. It was obvious, too, that in 1943 the dust should have been applied at least 10 days earlier for the best disease control.

Side applications of nitrate of soda in this test depressed yields slightly, but the results might have been different had the rainfall been uniformly distributed throughout the growing season.

The tribasic copper sulphate dust used in 1943 was definitely inferior to the cuprous oxide dust in preventing the foliage diseases. This inferiority was shown in the field tests where yield was the criterion and also in the greenhouse tests where the number of infections by *Alternaria solani* was the criterion. A comparison of the two materials in the laboratory by the spore toxicity test indicated that cuprous oxide was more toxic to spores of *A. solani* than tribasic copper sulphate in low dosages but these tests were inconclusive. The poor blending of the toxicant with the diluent undoubtedly accounted in part for the poor showing made by the tribasic copper sulphate dust in the field, but the results of the one greenhouse test with a re-mixed dust indicated that a properly blended tribasic copper sulphate dust was also less effective in preventing infection by *Alternaria solani* than was cuprous oxide dust. The average number of early blight lesions on plants treated with the re-mixed tribasic copper sulphate dust was 15.38, while on plants treated with cuprous oxide dust the average was 9.75. This evidence is not conclusive but it indicated that the toxicity of tribasic copper sulphate to *A. solani* is less than that of cuprous oxide. Horsfall and Heuberger (3) showed that the more finely divided forms of copper, as cuprous oxide, were more toxic to fungi than the less finely divided forms, as tribasic copper sulphate.

The most efficient use of metallic copper in the fungicide was in the form of cuprous oxide, because the cuprous oxide dust contained only 4.1 per cent metallic copper and the tribasic copper sulphate dust 6.0 per cent. In field performance, cuprous oxide dust in 1943 was superior to the tribasic copper sulphate dust and it was at least equally as good in previous years (2). Therefore, cuprous oxide should be recommended over tribasic copper sulphate during the war period when a shortage of copper exists.

SUMMARY

Cuprous oxide and tribasic copper sulphate dusts were compared as to their efficiency in controlling early blight, late blight, and Septoria leaf spot of tomatoes in southwest Virginia on plots receiving a basic fertilization only and on plots receiving supplemental applications of nitrate of soda. The toxicities of these two dusts to spores of *Alternaria solani* were also compared

in laboratory and greenhouse. The data obtained warrant the following conclusions:

1. Applications of the commercially mixed cuprous oxide dust available to growers in 1943 in southwest Virginia gave about 10 per cent higher yields than the commercially mixed tribasic copper sulphate dust.

2. In 1943, when August and September were abnormally dry, side applications of nitrate of soda depressed the yield of marketable fruit about 8 per cent.

3. The commercially mixed cuprous oxide dust was more effective than the tribasic copper sulphate dust in preventing infection of tomato foliage by *Alternaria solani* in the greenhouse. This difference was due in part to the toxicant in the tribasic copper sulphate dust not being sufficiently blended with the diluent.

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ADDITIONAL SPECIES OF LILIUM SUSCEPTIBLE TO LILY-ROSETTE VIRUS

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(Accepted for publication September 15, 1944)

Lily rosette,[3] or yellow flat in Ogilvie's original description,[4] is the most destructive virus disease of Easter lilies (*Lilium longiflorum* Thunb.). Lily growers cannot be too strongly warned against its introduction into production areas. Presumbaly of eastern Asiatic origin,[5] and best known from Bermuda,[4] rosette is reported also from England, Holland, and Java. Within the United States rosette is still restricted in distribution, and has been experimentally confirmed thus far only from one planting in southern Florida.

Little information on host range of the rosette virus is available, for Ogilvie[4] studied it in the Easter lily only. Rosette symptoms have been reported in *Lilium batemanniae* Wallace [*L. dauricum* var. *venustum* (Kunth) Wilson f. *batemanniae* (Wallace) Wilson] by Guterman[6] and in *L. auratum* Lindl. by K. M. Smith,[7] but neither of these writers mentions experimental transfers.

In the tests reported herein the rosette virus was originally isolated from Florida-grown Easter lilies. Transmissions were by the melon aphid, *Aphis gossypii* Glover, the only known vector, from naturally infected plants or from experimentally infected Easter lily seedlings. Leaves of diseased Easter lily seedlings have the characteristic downward curling 3 to 5 weeks after exposure, frequently become yellowed, and occasionally the upper leaves redden. Flowers, if produced on such plants, are noticeably smaller than normal and fail to open fully. Stem bulbils are developed freely in the lower leaf axils and occasionally in the upper leaf axils of blind plants. Early maturity is the rule. Infected bulbs are poorly developed and often decay, leaving only a cluster of small bulbils for propagation.

Virus-free seedlings of a number of species and varieties of *Lilium* were exposed to rosette by transfer of *Aphis gossypii* from affected Easter lilies. Three to 8 plants of each were inoculated, and return transfers to confirm susceptibility were made 2 to 11 months after exposure. Symptoms were

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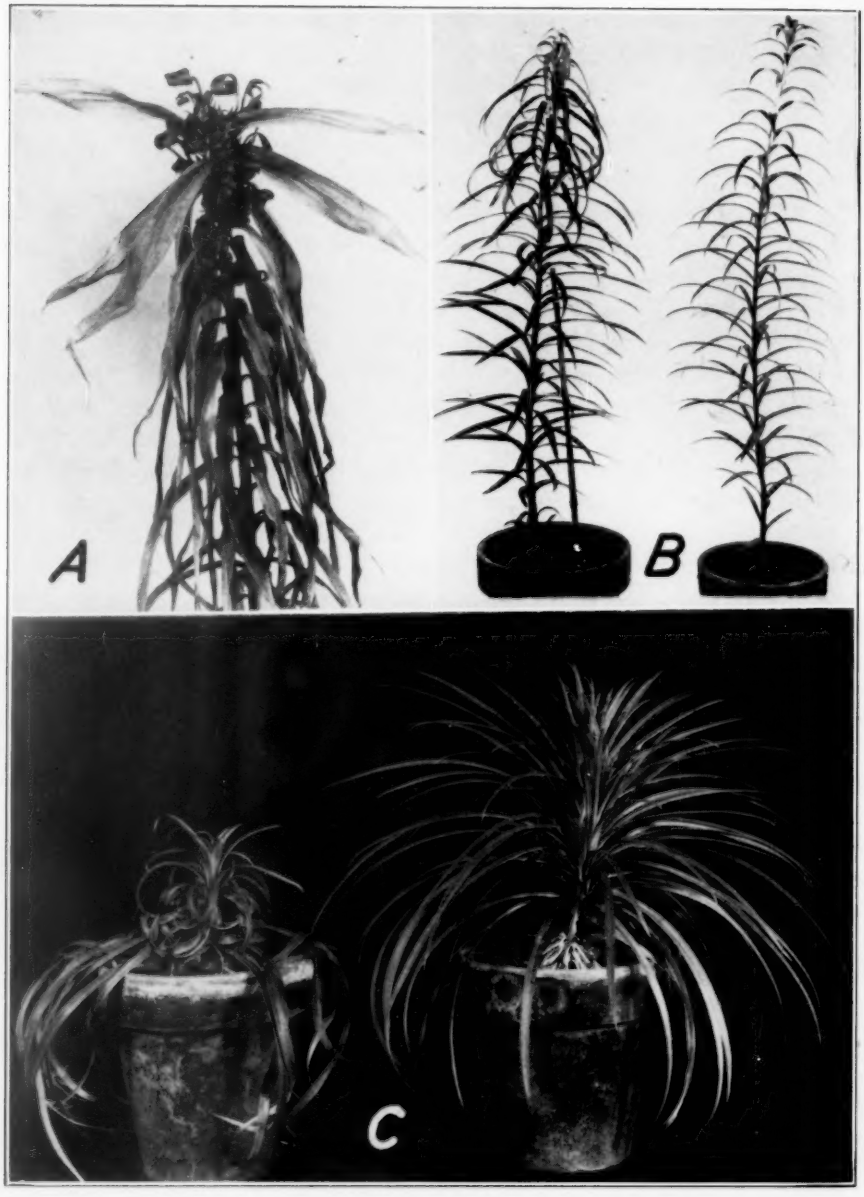

FIG. 1. A. Rosette in *Lilium sargentiae* inoculated April 12, photographed October 27, 1943, showing stem bulbils produced above the point of inoculation, and curled leaves developed from these bulbils. B. Initial symptoms of rosette in the same plant shown in A, photographed 29 days after inoculation (left) in comparison with uninoculated plant (right). C. Rosette in *L. formosanum* (left) with control (right), photographed April 29, 1944, in 5-inch pots 70 days after inoculation.

evident after about 3 weeks in the species *L. formosanum* Stapf (Fig. 1, C), *L. sargentiae* Wilson (Fig. 1, A, B), and *L. umbellatum* Hort. In these

species the disease can be diagnosed with reasonable accuracy. For brevity the symptoms observed in experimental plants are summarized in table 1. In these tests no other species showed as well-defined symptoms as the Easter lily. Many diseased species developed only slight dwarfing, irregular curling, or reddening, which in the field might be confused with the effects of root injury or poor cultural conditions.

There was considerable variation in symptom expression within species and varieties, and also in the same plant at different seasons. Therefore, all

TABLE 1.—*Species and varieties of Lilium experimentally infected with lily-rosette virus and confirmed by return transfer to Easter lily*

Species or variety	Symptoms[a]					
	Plants	Leaves			Flowers	
	Dwarfed	Curled	Yellowed	Reddened	Dwarfed	Lacking[b]
L. dauricum Ker-Gawl.	++	+	++	++	++	+
L. davidi Duchartre	+	+	+	+	−	+
L. davidi var. *willmottiae* Cotton & Grove	−	+	+	−	−	+
L. elegans Thunb.	+	+	+	−	++	−
L. formosanum Stapf	+	+++	−	−	+	−
L. henryi Baker	−	+	+	−	+	−
L. leucanthum Baker	+	+	+	+	−	+
L. longiflorum Thunb.	+++	+++	+++	+	++	+
L. myriophyllum var. *superbum* (Baker) Wilson	+	+	+	++	++	+
L. regale Wilson	−	+	+	+	−	+
L. sargentiae Wilson	+	++	++	+	++	+
L. speciosum Thunb.	−	+	+	+	+	−
L. umbellatum Hort.	+	+	+	++	++	−

[a] +, ++, +++ indicate increasing intensity of the symptom; − indicates that it was not observed.

[b] Absent from at least some of the plants in a test lot.

symptoms scored in table 1 are not necessarily evident in a given plant at a given time; and it is also possible that some effects scored as absent might be expressed if larger numbers of plants of these species were infected. Premature withering of leaves and of the upper stem were noted in *Lilium davidi* Duchartre and *L. leucanthum* Baker. There was no evidence of susceptibility of Clara Butt tulips to lily rosette in 5 tests (50 plants).

It is clear that lily rosette is of considerable potential importance in the case of various garden lilies. Moreover, many of these lilies, although worthless for ornament when affected, fail to show sufficiently clear symptoms to permit accurate diagnosis, and could serve as unsuspected reservoirs of the rosette virus.

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PATHOGENICITY OF ISOLATES OF RHIZOCTONIA SOLANI FROM POTATOES

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While studying the pathogenicity of strains of *Rhizoctonia solani* on bunch snapbeans, there were included in one of the inoculation tests 11 isolates from Irish potatoes. Of these, 6 were from stem lesions and 5 from sclerotia on the tubers. None of them was pathogenic to beans. This suggested two questions, answers to which seem important. Seed potatoes coming into Louisiana frequently have on them large numbers of sclerotia of *Rhizoctonia*. No losses have been observed following the planting of such potatoes and consequently the treatment of tubers with fungicides that would destroy the sclerotia has never been recommended. On the other hand, many Louisiana crops are severely attacked by *Rhizoctonia*. The questions for which solutions seemed desirable were: (1) Do the sclerotia on potato tubers brought into Louisiana for seed purposes produce strains that attack the stems of young potato plants? (2) Do these strains attack other plants in Louisiana and does the planting of tubers with sclerotia add undesirable pathogenic strains to the soil? To answer these questions a rather large number of isolates obtained from sclerotia on tubers and from lesions on potato stems were tested on bean and potato stems in comparison with isolates from other hosts.

The isolates used included 97 cultures from sclerotia on Irish potato tubers from 71 cars of seed potatoes shipped into Louisiana in 1941 from Nebraska, North Dakota, Maine, and Colorado; 4 cultures from sclerotia on tubers raised in Louisiana; 3 cultures from Dr. Kotila who isolated them from sclerotia on tubers; 29 cultures from stem lesions on Irish potato plants in Louisiana; 2 cultures from beans; and 2 cultures from sugarbeets which had been found pathogenic in previous tests.

In the inoculation tests with beans the plants were grown in sterilized soil, inoculated with the cultures of *Rhizoctonia*, and the severity of infection on the stems was noted at the end of about 20 days. For the inoculation tests with Irish potatoes, clean tubers were sterilized for 5 minutes in acidified bichloride of mercury. These were planted in sterilized soil and the inoculum was then added. Pathogenicity was measured by the size of the lesions on the young stems. With beans two tests of two replications of 20 seeds each were made with each culture, except for a few cultures that were tested only once. In most instances the results with Irish potatoes represent the average of 3 tests, each with 8 or more plants.

The results of inoculating beans are in table 1. Only 3 of the 101 cultures from sclerotia from potato tubers attacked beans and these were only slightly pathogenic. Of the 29 isolates obtained from lesions on potato stems, 9 were strongly pathogenic, causing deep lesions on the bean stems,

TABLE 1.—*Relative pathogenicity on beans of 101 cultures from sclerotia on Irish potatoes and 29 cultures from lesions on potato stems*[a]

Source of cultures	Number of cultures			
	Non-pathogenic	Slightly pathogenic	Strongly pathogenic	Total
Sclerotial isolates				
Colorado	5	.....	.....	5
Louisiana	4	.....	.....	4
Maine	5	.....	.....	5
Michigan	1	.....	.....	1
Nebraska	58	2	.....	60
North Dakota	25	.....	.....	25
Unknown	.....	1	.....	1
Total	98	3	.....	101
Stem lesion isolates				
Louisiana	19	1	9	29

[a] Two isolates from bean stem lesions and 2 isolates from sugarbeets included as check cultures in these tests were strongly pathogenic to beans.

one was slightly pathogenic, and 19 were nonpathogenic. The 2 bean isolates and 2 sugar-beet isolates used as check cultures were strongly pathogenic in the tests. The control plants were not infected.

In table 2 are the results of the tests with young potato plants. Seventeen of the 70 isolates from potatoes were not pathogenic, 35 were slightly pathogenic, 13 were moderately pathogenic, and 5 were strongly pathogenic. The 2 cultures from beans and the 2 from sugar beets used as checks were moderately pathogenic to potato plants. The control pots were free of infection.

Of the 3 cultures from sclerotia on tubers which had been pathogenic to beans, one was not pathogenic to Irish potatoes and two were only slightly pathogenic.

It would seem from these studies that the sclerotia present on Irish potato tubers have little effect on the Rhizoctonia solani potential in the soil. On the other hand a number of isolates from potato stem lesions were patho-

TABLE 2.—*Relative pathogenicity of 70 cultures of Rhizoctonia isolated from sclerotia on Irish potato tubers*[a]

Source of cultures	Number of cultures				
	Non-pathogenic	Slightly pathogenic	Moderately pathogenic	Strongly pathogenic	Total
Colorado	1	1	1	.....	3
Louisiana	1	2	.....	.....	3
Maine	.....	5	1	.....	6
Michigan	1	.....	.....	.....	1
Nebraska	9	15	9	4	37
North Dakota	4	12	2	1	19
Unknown	1	.....	.....	.....	1
Total	17	35	13	5	70

[a] Two isolates from bean stem lesions and 2 isolates from sugar beets included as check cultures in these tests were moderately pathogenic to Irish potatoes.

genic to beans, and it is possible that a potato crop preceding beans may have some effect on the perpetuation or increase of strains of the fungus pathogenic to beans. It is probable that the stem lesions from which these isolates were obtained resulted from infection with soil *Rhizoctonias* and did not come from sclerotia or mycelium on the seed tubers.

The fact that 70 per cent of the sclerotial isolates were either nonpathogenic or only slightly pathogenic to Irish potatoes and only 7 per cent were strongly pathogenic indicates that most of the sclerotia on the tubers play relatively little part in increasing the damage done by *Rhizoctonia solani* to Irish potatoes. These results agree with those of Sanford[1] who showed that the majority of cultures from sclerotia were nonpathogenic or of marginal pathogenicity. This may help to explain the erratic results obtained by many investigators in seed treatment tests on potato tubers for the control of *R. solani*.

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LEAF SCALD OF SUGAR CANE IN BRAZIL

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Since March, 1943, studies have been in progress on a disease of sugar cane which has been present for several years at the Estacão Experimental de Cana de Açucar and on neighboring sugar-cane properties in Piracicaba, State of São Paulo, Brazil. The disease causes an intense chlorosis of the top leaves of the affected plants and has therefore been referred to as sugar-cane albinism. Preliminary investigations showed that the disease was transmitted from the seed pieces and that every bud of a diseased stalk invariably gave rise to a diseased plant.

Field and greenhouse observations of affected plants have led us to conclude that the albino condition of the top leaves is only a final stage in the development of the disease, and that in the primary stage there are less conspicuous chlorotic streaks with sharply-defined borders, 1–2 mm. wide, which run from the tip to the base of the leaf and continue down on the leaf sheath. Later, as the streaks broaden from the tip downward, there is a progessive withering of the tissues in the same direction, giving a final scalded appearance to the leaves. The severely affected stalks very commonly produce lateral shoots and on these the developing leaves very quickly show the typical chlorotic streaks. In the more advanced stages of the disease, especially on the more susceptible varieties, the top leaves on the affected stalks become completely chlorotic. When a diseased stalk that is approaching maturity is split and examined, a light red discoloration of the fibrovascular bundles, mainly in the nodal regions, is frequently observed. This discoloration seems to be more pronounced in susceptible varieties such as C.P. 29/320.

A comparison of the symptoms with descriptions of other sugar-cane diseases not known to occur in Brazil has shown that the Brazilian disease agrees very closely with what is found in the literature for the chronic phase of leaf scald, a bacterial disease caused by *Bacterium albilineans* Ashby. The identity of the disease has been confirmed by microscopic examinations and by cultures. Leaf scald is distributed in the Eastern Hemisphere and Hawaii, but up to this time has not been reported on the mainland of the Western Hemisphere.

Microscopical examinations have shown that the bacteria are present in the xylem elements of the fibrovascular bundles in the affected stalks as well as in the leaf streaks. Pure cultures have been obtained by first making tissue plantings in a 25 per cent sugar-cane juice and peptone medium in tubes and then making dilution plates after about 8 days from those tubes that became slightly turbid. An incubation temperature of 25° C. was used.

The cultural behavior and the physiological and morphological characters of the Brazilian organism agree closely with those of *Phytomonas albi-*

lineans Ashby as they have been presented by Martin, Carpenter and Weller,[1] Wilbrink,[2] and North.[3]

The bacterium is a slender, rod-shaped organism, Gram-negative, forming chains and threads in culture but not producing spores. It is motile, though the flagellae have not as yet been successfully stained by Zettnof's method. In agar plates (after 5–8 days), it forms small to minute colonies with glistening surface and entire margins, viscid and buff yellow. Broth becomes turbid and a filament forms which is fixed to the bottom of the tube. Other physiological reactions include: fair growth in sugar but no fermentation; fair growth in peptone water in presence of carbohydrates; no hydrolysis of starch; hydrogen sulphide, indol, and ammonia production negative; no reduction of nitrates; no liquefaction of gelatin; no change in milk.

TABLE 1.—*Inoculation tests with the bacterium associated with the albinism of sugar cane in Brazil*

Culture	Origin of culture	Inoculation date	Seed pieces planted		Plants infected after 2 months		Infection
			Inoc.	Check	Inoc.	Check	
			No.	*No.*	*No.*	*No.*	*Per cent*
27	C.P. 29/320	Apr. 20, 1944	31	15	16	0	51.6
48	P.O.J. 2878	Aug. 5, 1943	21	9	2	0	9.5
49	C.P. 29/320	do	14	5	6	0	42.8
55	Co. 281	do	14	6	10	0	71.4
77	Co. 290	Oct. 29, 1943	10	5	10	0	100.0

To prove that the bacterial cultures isolated from affected cane plants were pathogenic and would produce the symptoms observed in the fields, seed pieces were inoculated under controlled conditions with four cultures, Nos. 27, 48, 49, and 55. Cultures were isolated from stalks of different varieties from several places in Piracicaba, the first in March and the others in July, 1943. Single-eye seed pieces were selected. The inocula which consisted of suspensions of bacteria from pure cultures in sterile water were smeared on the cut ends of the seed pieces just before planting. Seed pieces of the variety Co. 290 were used although it is not the most desirable variety for inoculation tests due to its relative resistance or tolerance to the disease. The results from the inoculation tests are in table 1.

In the tests, the shoots growing from seed pieces inoculated with cultures 48, 49, and 55 came up in an apparently healthy condition. One month after the inoculations were made, however, a withering of the tips of the basal leaves was noted. This withering progressed downward to the bases

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of the leaves and the other portions of the plants appeared much paler than the control plants. Some of the plants wilted and died, appearing to have the acute stage of the disease. Most of the plants, however, had the general appearance of recovering to a certain extent with the disease perhaps in a dormant condition for a period of 1 or 2 months. After that period, the typical chlorotic streaks, characteristic of the disease, were observed. The inoculations with culture 27 were made during cool weather and the shoots took a longer time to come up. Several of these appeared above the ground in a completely chlorotic condition, though most of them did not become completely chlorotic for about 5 months.

The bacterium was reisolated from Co. 290 plants inoculated with culture 55 and infection was obtained when one of the isolates (culture 77) was inoculated on C.P. 29/291. (See table 1.)

As the symptoms of the Brazilian disease agree with those described for leaf scald and as the morphological, physiological, and cultural characters of the causal organism agree with those of *Phytomonas albilineans* Ashby, it is believed that the evidence is sufficient to consider that the Brazilian disease is identical with the leaf scald of the Eastern Hemisphere.

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PHYTOPATHOLOGICAL NOTES

Phytophthora Wilt and Stem Canker of Cinchona.—Seedling blights of cinchona, caused by *Phytophthora* spp., have plagued growers in many parts of the eastern hemisphere. Observations by growers indicate that similar diseases will be of economic importance in the establishment of a cinchona bark industry in the American tropics.

Celino,[1] in 1934, reported that *Phytophthora faberi* Maubl. (synonymous with *P. palmivora* Butl.[2]) was causing a severe blight of seedlings of *Cinchona calisaya* var. *ledgeriana* Howard, *C. hybrida* Hort., and *C. succirubra* Pavon in the Philippines. Celino considered the disease an important factor in the growing of cinchona. In 1935 Kheswalla[3] reported a similar serious disease, caused by *P. palmivora,* on seedling *C. ledgeriana* Moens in India. Sawada,[4] in 1936, reported a disease in Formosa on cinchona seedlings with symptoms differing slightly from those reported from the Philippines and India. As described by him, the infection apparently occurred on the stem with the *Phytophthora* subsequently spreading upward to the tip of the plant. In India and the Philippines the infection court was apparently the tip of the plant. The Formosan *Phytophthora* was considered a new species and was described as *P. cinchonae* Saw. Seedling blights of cinchona caused by *Phytophthora* have not been reported from Java, although Hartley[5] reported *P. faberi* (*P. palmivora*) on other hosts. Hartley stated orally that the forms of *P. palmivora* he studied from several hosts in Java differed from most of the isolates of *P. palmivora* that he had observed from other parts of the world. One outstanding difference was the sparse sporulation of the Java forms. They nevertheless caused similar disease symptoms and probably were referable to *P. palmivora.*

As reported in the literature, the Phytophthora diseases mentioned above apparently affect seedlings only. However, a disease or diseases with similar symptoms were observed by the authors in Central and South America on seedlings, grafted stock, and mature and semi-mature plantation trees. A similar disease was also observed in Puerto Rico on seedlings only. The infection court seems to be the petiole region of the tender new leaves or the succulent stem tissue. In the early stages on the leaves the fungus causes brown, necrotic spots with indefinite margins. From initial infections at the base of one or more leaf blades the fungus may grow through the entire length of the petiole and enter the stem. On seedlings the entire tree is killed as the pathogen spreads downward through the cambial region of the stem.

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[3] Kheswalla, K. F. Seedling blight of *Cinchona ledgeriana* Moens caused by *Phytophthora palmivora* Butl. in the Darjeeling district. Indian Jour. Agr. Sci. 5: 485–495. 1935.

[4] Sawada, K. [Phytophthora blight of cinchona seedlings occurring in Formosa, caused by *Phytophthora cinchonae* Saw. n. sp.] Formosan Agr. Rev. 32: 326–346. 1936.

[5] Hartley, Carl. [Diseases of Cacao.] Instituut voor Plantenziekten, Dept. van Landbouw, Nijverheid en Handel Bull. 19. 1924.

On older grafted stock and seedling plantation trees the symptoms of infection are elongated, slightly sunken cankers and dieback of the more succulent branches. In some cases on older trees the terminal portion of every branch is killed, leaving the tree with clusters of brownish-black dead leaves.

This disease was observed in Guatemala at several locations on seedling *Cinchona pubescens* Vahl (called *C. succirubra* locally), *C. officinalis* L., and the Ledger form of *C. officinalis*. It was also noted on the Ledger form grafted on *C. pubescens* in nursery and plantations and on *C. officinalis* and *C. pubescens* in plantations. The disease was observed in Puerto Rico on nursery stock of the Ledger form of *C. officinalis*. Stem cankers on both *C. pubescens* and *C. pitayensis* Wedd. and branch dieback on *C. pubescens*

FIG. 1. A. Dieback of branch tip of *Cinchona pubescens* in Colombia. B. Tip dieback and infection of adjacent leaves induced by inoculation on the Ledger variety of *C. officinalis*.

were observed in Colombia on cinchona trees growing wild in forest stands (Fig. 1, A).

In Peru, seedlings of the Ledger form of *C. officinalis* and some cinchona hybrids as well as plantation stock of the Ledger form were diseased with symptoms similar to those observed in Guatemala, Colombia, and Puerto Rico. From part of the Peruvian seedling material a *Phytophthora,* tentatively identified as *P. parasitica* Dast., was isolated from leaf blade, petiole, and stem material. This is apparently the first recorded occurrence of this species on cinchona as well as the first reported Phytophthora blight of cinchona in the western hemisphere. Two isolates available for study were characterized by optimum growth temperatures of 25° C. but were able to grow at temperatures as high as 37.5° C. Eight-day-old cultures on cornmeal agar produced prominently papillate sporangia, averaging 30×24 μ, on branching sporangiophores 2 days after the plates were flooded with water.

The pathogenicity of one of the 2 isolates has been demonstrated by inoculation on 18-inch potted plants of the Ledger form of *C. officinalis* grown from seed of Philippine origin. Six days after inoculation, 3 of 4 stem inoculations with mycelium introduced into a small cut to the cambium had produced girdling cankers, while 2 check incisions in which sterile agar was introduced remained healthy. Four bud tips inoculated without wounding by placing mycelium in agar blocks in contact with them became infected, and dieback was produced (Fig. 1, B); 2 check treatments remained healthy. Twelve mature, unwounded leaves were inoculated by placing mycelium growing in agar blocks in contact with the upper surface. Five infections resulted with symptoms typical of leaf infection as it occurred in the field. Infections did not spread through the petiole to the stem, however, because the leaves dropped before this occurred. Six leaves on which sterile agar was placed remained uninfected. The fungus was recovered from one of the 5 leaf infections, 2 of the 4 tip infections, and at a distance of $\frac{1}{2}$ inch or more from the point of inoculation from all 4 of the stem cankers. One of the 4 stem inoculations did not result in a girdling canker. The tree, however, suddenly wilted and lost its leaves 11 days after inoculation. The canker had not girdled the stem, although the *Phytophthora* was still alive and was recovered from the margin of the infection. Dissection and culturing of the entire tree demonstrated that the cortical tissue from root to tip was infected. At the time of culturing only slight discoloration gave indication of this infection.

Some control apparently was obtained in Peru by eradicating wilted seedlings from the beds. In Guatemala, control also was secured and considerable material salvaged by cutting back the tops of infected grafted stock to well below the dead tip. The cortical infection that resulted in the single inoculated tree might give indication that cutting back, unless done very early and severely, may not give control. Experience with *P. parasitica* in the United States would seem to indicate that control could be obtained by regular application of a copper spray containing a good sticker. Wherever the disease was observed, considerable evidence of host resistance of individuals and of certain clonal lines was apparent. It appears likely that the selection of resistant clones of high alkaloid content will be possible.—BOWEN S. CRANDALL, Associate Pathologist, and WILLIAM C. DAVIS, Pathologist, Division of Latin American Agriculture, Office of Foreign Agricultural Relations, in cooperation with Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Crater Rot and Blotch of Celery, a New Aspect of Soft Rot Caused by Erwinia carotovora.—*Erwinia carotovora* has been found to produce conspicuous and destructive symptoms other than heart rot which result in large losses in commercial celery fields in the Delta region in California. Small spots, at first watery, then straw-colored to brownish, appear near the base of

the petioles (Fig. 1, B), and later become sunken, dark-brown, with sharply defined edges (Fig. 1, D). In some cases only superficial lesions are induced (Fig. 1, C), resulting in a blotchy appearance, in contradistinction to the deeper, soft watery rot. Later, the affected tissues dry out and collapse resulting in concave, crater-like depressions.

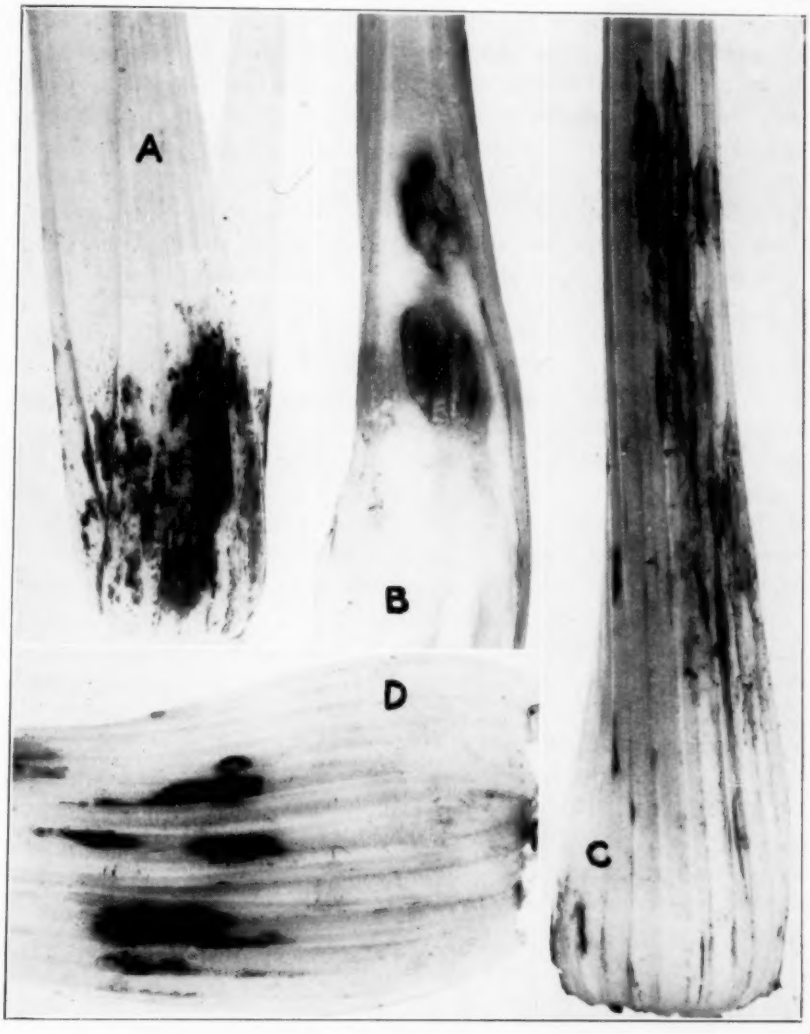

FIG. 1. A. Crater rot and blotch of celery caused by *Erwinia carotovora* on Golden Self Bleaching celery grown in peat soil, before ridging. B. Crater rot on ventral side of a petiole. Golden Self Bleaching variety. Plants were ridged and heavily irrigated. C. Blotch disease of celery (*E. carotovora*) on dorsal side of a petiole of Golden Self Bleaching celery, from plants that were not ridged. D. Dorsal side of a petiole of Golden Self Bleaching celery showing crater rot. Collapse of the tissues caused deeply sunken depressions with sharp edges. Celery was grown in peat soil and heavily irrigated.

In some years losses from crater rot and blotch are severe, since badly diseased plants cannot be shipped and stripping off of the outer larger peti-

oles is necessary. The practice of leaving diseased stalks in the field results in heavy infestation of the soil with the crater-rot bacteria and may account for the increase of the disease when celery is repeatedly grown in the same field.—P. A. ARK, University of California, Berkeley 4, California.

Susceptibility to Black Root Rot of Apple Trees Having Various Root and Top Combinations.—In continuation of previously reported work on the susceptibility of own-rooted trees to black root rot[1] another experiment is here reported where trees having various root and top combinations were tested for their susceptibility to the black-root-rot fungus (*Xylaria mali* Fromme).

TABLE 1.—*Percentages*[a] *of roots of various apple varieties infected by Xylaria mali, averages for four seasons*

Root variety	Trees infected			
	Cortex infection only	Deep infection	Total infection	Av. size of lesion
	Per cent	*Per cent*	*Per cent*	*mm.*
Arkansas	12.5	83.3	95.8	29.6
Ben Davis	41.7	56.2	97.9	28.6
Delicious	11.4	74.4	85.8	32.3
Fallawater	17.1	72.0	89.1	32.4
McIntosh	9.2	84.4	93.6	26.6
Nero	30.1	61.6	91.7	25.7
Northern Spy	11.6	74.2	85.8	32.4
Opalescent	9.9	72.8	82.7	25.9
Perkins	16.6	66.7	83.3	35.7
Red Astrachan	12.5	75.0	87.5	22.0
Rome Beauty	15.9	67.2	83.1	24.4
Smith Cider	16.5	70.5	87.0	30.1
Starking	22.5	77.5	100.0	34.7
Stayman Winesap	25.3	66.4	91.7	29.7
Summer Rambo	26.4	67.4	93.8	27.0
Wealthy	36.4	58.2	94.6	28.1
Williams	32.7	55.3	88.0	26.8
Winesap	25.0	68.7	93.7	32.3
Winter Banana	12.5	75.0	87.5	19.7
Yellow Transparent	17.4	69.3	86.7	25.2
York Imperial	22.6	63.6	86.2	26.1

[a] All figures represent averages for 1938 through 1941.

In 1937 apple trees 2 years from the graft were set 18 inches apart in rows 4 feet wide. Inoculation was begun in 1937 and continued each year for 4 years.

The trees were either own-rooted or consisted of varieties worked on an own-rooted tree, so that top and root were of known varieties. A separate record of the percentage of infection and size of lesion was made for each root and top combination. Since there were no outstanding differences in susceptibility because of top influence, the various root and top combinations were grouped according to root variety, of which there were 21, as given in

[1] Cooley, J. S. Factors affecting distribution and severity of black root rot of apple trees. Jour. Agr. Res. [U.S.] 65: 299–311. 1942.

table 1. In the case of McIntosh variety, for instance, a combination was made of infection results with Red Rome on McIntosh, Starking on McIntosh, and own-rooted McIntosh. The combined number of trees in each plot was from 5 to 28, usually about 15. The infection percentages and size of lesions were averaged for 1938, 1939, 1940, and 1941. A high percentage of deep infections will be noted, representing an average of four years' inoculation results. Since the data show a high percentage of positive results, they should have some weight even if they are not based on a large number of individuals. There were some differences in susceptibility (Table 1) as in other experiments on own-rooted varieties previously reported,[1] but unfortunately there was no outstanding resistance in any of the 21 varieties of roots tested.—J. S. COOLEY, Senior Pathologist, Division of Fruits and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Breeding Oats to Combine Resistance to Race 45 and Other Races of Crown Rust Common in Arkansas.—The current, 1944, season marks the first year in which race 45 of crown rust, *Puccinia coronata,* has been found in such abundance on oats as to constitute a distinct hazard to future oat crops in Arkansas in addition to reducing yields considerably during the current season.

Since 1937, when race 45 was first detected in this State and when Moore, Downie, and Murphy[1] described it, this race has been of minor importance until 1944. It has usually appeared late and seldom in greater abundance than 5 to 10 per cent of leaf area on Bond and its hybrids. Race 45 has been identified by inoculation of the differential hosts used by Murphy. In 1944 it appeared on spring-planted Bond shortly after the boot stage and before anthesis. By the time of the early milk stage fully 30 per cent of the leaf area was infected, and probably more damage was caused than the percentage figure indicates. At that stage most culms had only two green leaves left, the lower leaves having died earlier, apparently as a result of Helminthosporium attacks combined with adverse growing conditions.

On winter oats the effect of race 45 was not nearly so severe because it did not appear in abundance until most of the oats had reached the dough stage. However, its influence on most varieties of winter oats grown at the University Farm, Fayetteville, was not insignificant since the rust was present on Bond hybrids in considerable amounts prior to the maturity of the grain.

Without much doubt, race 45 was also present on most other varieties which possessed no Bond parentage. In general, Victoria hybrids showed about the same reaction to this race as Victoria does to race 1, with perhaps a greater tendency to produce large pustules, although these were still accompanied by some chlorosis and necrosis. The word "perhaps" is used

[1] Moore, M. B., A. R. Downie, and H. C. Murphy. A new race of crown rust that attacks Bond oats. (Abstr.) Phytopath. 28: 16–17. 1938.

because other race or races were present in abundance, judging by the amount of rust on varieties that are susceptible to one or more races other than race 45. For example, on June 8, Markton was fully 90 per cent infected when Bond averaged about 30 per cent.

Shortly after race 45 was first found in the State, a search was begun for varieties that would be resistant to this race as well as to the races previously found here. One variety, Mutica Ukraina, showed a type of resistance approaching immunity to race 45. Dr. H. C. Murphy[2] had previously called attention to the high crown rust resistance of this variety, including resistance to races 33 and 34 (published as 34 and 35). Since then, it has been moderately susceptible to a strain of race 1. This strain produces comparatively few infections on this variety and these are characterized mostly by small pustules under natural as well as artificial conditions of infection.

While Mutica Ukraina has little agronomic value, as Dr. Murphy has shown, its potential value for breeding was recognized and it was used extentively by the writer as one of the parents in hybridization work in 1938 and later.

Out of this crossing there is now available one hybrid with a high degree of resistance not only to race 45 but to races 1, 7, and several others not so common as these two in Arkansas. It is the only one in a varietal test including 23 varieties and selections which was not severely rusted in 1944.

Parentage of the hybrid is Mutica Ukraina by R3–II. R3–II was derived from a cross made by the writer and L. M. Weetman in the winter of 1936–37, Coker 32–1 × (Victoria × Richland C.I. 3313). The hybrid thus has two different sources of crown rust resistance, Victoria and Mutica Ukraina, and two different sources of smut resistance, Coker 32–1 and Victoria. At present it is known only by its breeding number 674 (2)–39f. Its agronomic characters, which are seemingly quite promising for a spring oat, will be described elsewhere.—H. R. ROSEN, Agricultural Experiment Station, University of Arkansas, Fayetteville, Arkansas.

A Penicillium Disease of Soybeans.—During the winter of 1943–1944, numerous lots of soybeans being tested for percentage of germination by the Ohio Seed Improvement Association were examined for the presence of diseases. On many of the plants two types of lesions were common. One type of lesion, found on both cotyledons and hypocotyls, consisted of a sunken, irregular dark brown spot. Another type of lesion was a soft, water-soaked, slightly dark spot on the hypocotyl of germinating seedlings. Similar lesions on the cotyledons of germinating seedlings were unusually large, frequently involving an entire cotyledon which would be a soft light gray mass of decayed tissue.

A species of *Penicillium* was isolated rather consistently from both types of lesions. Cultures of the fungus on both broth and agar were used to inoculate several varieties of soybeans. The results were very striking.

[2] Murphy, H. C. Making new strains of oats resistant to crown rust by selection and hybridization. Iowa Agr. Expt. Sta. Ann. Rept. 1935–1936: 98–100. 1936.

Inoculated seeds germinated much more slowly than the checks. Those inoculated seedlings which emerged were stunted and the leaves were distorted, being about half the size of normal leaves (Fig. 1). Some stunted

FIG. 1. Symptoms produced by *Penicillium* sp. on soybeans. Rows 1 and 4, checks. Rows 2, 3, 5, and 6, inoculated. Photograph by F. H. Norris.

plants developed lateral buds which resulted in a fasciation effect. The base of the hypocotyl and the cotyledonary leaves of the infected plants had numerous lesions similar to those observed previously on plants in the germinator. On check plants these lesions were almost entirely absent. When cultured, the fungus was readily recovered.

Varieties of soybeans were given a preliminary test of relative susceptibility. Percentages of emergence were as follows:

Variety	*Inoculated*	*Checks*
Chief	60	100
Dunfield	8	76
Earlyana	36	92
Lincoln	68	96
Manchu	64	96
Mingo	12	96
Richland	56	96
Scioto	28	92

The species of *Penicillium* has not been identified. Studies are under way to determine the percentage of infection this fungus causes under field conditions.—M. R. HARRIS, U. S. Department of Agriculture, Emergency Plant Disease Prevention Survey and C. W. ELLETT, Botany Department, The Ohio State University.

Verticillium Wilt and Die-back of Viburnum.[1]—A disease manifested by an unthrifty condition, die-back, and frequently death of Viburnum shrubs was first observed at Lafayette, Indiana, in 1940. *Verticillium albo-atrum* Rienke and Berth. was isolated from the diseased shrubs, and the capacity of isolates from chrysanthemum, peppermint (*Mentha piperita* L. var. *vulgaris* Sole), and *Viburnum lantana* L. to produce this disease was studied.

[1] Journal Paper Number 174, of the Purdue University Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

The new growth of infected canes is stunted, the leaves flag, become light green, and then brown when the branches and canes die. Infected canes usually die during the growing season and the brown leaves remain attached until late in the winter. Often only one or a few canes of a shrub may have the symptoms. Sometimes only the branches on one side of a cane are affected. Infected shrubs may succumb in one or two years, or may live in an unthrifty condition for several years. Brown sectors or continuous bands of discolored wood occur and frequently extend from the base to the tip of the infected canes. Only those branches that are contiguous with discolored xylem of a unilaterally infected cane die or display symptoms of the disease. Finally such canes become completely infected and die.

The causal fungus was isolated readily from discolored wood, but not from apparently healthy tissue taken from the distal end of canes.

Verticillium albo-atrum, the causal fungus, was isolated consistently in apparently pure culture from the discolored wood of naturally infected *Viburnum lantana* and *V. tomentosum* Thunb. The fungus grew well on potato-dextrose agar and appeared to be typical of *V. albo-atrum*. Many of the cultures produced variants (saltants), as described for *Verticillium* by Presley,[2] Rudolph,[3] Thompkins and Ark,[4] and Tilford and Runnels.[5] Cultures isolated from *Viburnum* spp. appeared to be similar to those from chrysanthemum and peppermint in color and in production of pseudo-sclerotia and variant sectors. All cultures formed variants that differed greatly in color, production of pseudo-sclerotia, and conidial sporulation.

Presley[2] and Rudolph[3] showed that the production of pseudo-sclerotia by cultures of *V. albo-atrum* was extremely variable and that *V. dahliae* Kleb. in this and other respects did not differ from *V. albo-atrum*. Tilford and Runnels[5] although they recognized that the cultures of *Verticillium* that they isolated from chrysanthemum were unstable, referred them to *V. dahliae*, since the initial cultures on synthetic medium produced abundant pseudo-sclerotia. The writer concurs with Rudolph[3] in considering the production of pseudo-sclerotia an undependable specific character and prefers to refer this species attacking *Viburnum* to *Verticillium albo-atrum*.

Shrubs of *Viburnum lantana* about 24 inches high were planted about the middle of March, 1941, in 12-quart metal pails that had been lined with a coating of tar. Five weeks after planting, four shrubs were inoculated with an isolate from *V. lantana*, two with an isolate from chrysanthemum, and two with an isolate from peppermint. The inoculations were made by placing one half-pint of a 3-week-old culture of the fungus growing on a mixture of equal parts of corn meal and sand in the soil close to the roots. Four shrubs were treated similarly with sterile medium. The shrubs were

[2] Presley, John T. Saltants from a monosporic culture of *Verticillium albo-atrum*. Phytopath. 31:1135–1139. 1941.

[3] Rudolph, B. A. *Verticillium hydromycosis*. Hilgardia 5: 197–353. 1931.

[4] Thompkins, C. M. and P. A. Ark. Verticillium wilt of strawflower. Phytopath. 31: 1130–1134. 1941.

[5] Tilford, Paul E. and Harmon A. Runnels. Verticillium wilt of chrysanthemums and its control. Ohio Agric. Exp. Stat. Bull. 630. 1942.

grown in a greenhouse during the spring of 1941, then were placed outdoors during the summer and returned to the greenhouse in October. In May, 1942, the surviving shrubs were placed outdoors and remained there until the next year. The buckets, when outdoors, were buried in the ground to within two inches of the top, and were kept well watered.

One plant that had been inoculated with the isolate from *V. lantana* showed symptoms of "Verticillium hydromycosis" 8 months and the remaining three plants 13 months after inoculation. The two shrubs that were inoculated with the isolate from chrysanthemum developed typical symptoms 6 and 9 months after inoculation. One of the shrubs inoculated with the isolate from peppermint developed typical symptoms of "Verticillium hydromycosis" 25 months after inoculation and died 4 months later. The other shrub inoculated with this isolate died from an undetermined cause a few months after inoculation. The four control shrubs remained healthy during the test. The fungus was reisolated from all of the infected shrubs and appeared to be similar to the original cultures.

The results of inoculating *Viburnum lantana* with the three isolates of *Verticillium albo-atrum* indicated that possibly the isolate from peppermint was less pathogenic than the isolates from chrysanthemum and *V. lantana*. The three isolates were tested on chrysanthemum, variety Amoskeag; eggplant, variety N. H. Hybrid; pepper, variety California Wonder; and pimiento, variety Sweet Meat Glory, to determine if they differ in pathogenicity to these hosts. Four-week-old cultures of the isolates growing on a mixture of equal parts of corn meal and sand were incorporated in flats-of-soil that had been steam-sterilized. Eight eggplant, pepper, and pimiento plants, about one month old and between $2\frac{1}{2}$ and $3\frac{1}{2}$ inches high, and 8 small chrysanthemum plants were planted in the infested soil in April and allowed to grow until July. A like number of the plants also were planted for "controls" in soil in which similar, but sterile, medium was incorporated.

The eggplants in the soil infested with the isolates from chrysanthemum and *Viburnum lantana* were stunted and approximately half of the plants were partially defoliated and the remainder defoliated, except for the terminal leaves, 6 weeks after transplanting. The eggplants in the soil that was infested with the isolate from peppermint showed symptoms of "Verticillium hydromycosis" but were not stunted nor defoliated as severely as with the other two isolates. Cultures of *Verticillium albo-atrum* that were reisolated from a number of the infected eggplants appeared to be similar to the original isolates. The inoculated chrysanthemum, pepper, and pimiento plants remained uninfected. Control plants of the eggplant were not infected.

The results, although not conclusive, are suggestive of variation in pathogenicity in the cultures of *V. albo-atrum* studied. This is similar to the results of Tilford and Runnels who reported "forms" of *Verticillium* differing in pathogenicity on chrysanthemum.—R. C. BAINES, California Department of Agriculture, Sacramento, California.